A Field Evaluation of Point-of-Care Urinalysis Drug Testing Devices

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A field study was performed at two police agencies to evaluate the utility and accuracy of 5-selected point-of-care (POC) urinalysis drug testing devices when testing driving under the influence (DUI) arrestees. The devices selected for evaluation were AccuSign®, Rapid Drug Screen®, TesTcup–5®, TesTstik® and Triage®. Standard workplace screening drug concentrations were used and samples were tested for marijuana, cocaine metabolites, amphetamine(s), opiates and PCP (except opiates 300 ng/mL). Four hundred arrestees were recruited at each site, informed consent was obtained and a urine specimens were collected from each subjects for testing. Police officers preformed testing with one device and trained technicians performed testing with the other four devices. The device used by the officers rotated on a random basis. All positive and 5% of the negative samples were retested in a laboratory by mass spectrometry. Laboratory cutoff concentrations were: carboxy-THC (4 ng/mL); benzoylecgonine (50 ng/mL); amphetamines (100 ng/mL); opiates (50 ng/mL); and PCP (5 ng/ml).

Approximately 1/3 of the subjects tested positive. No randomly selected sample that tested negative on the devices tested positive at the laboratory. A false positive was defined as testing positive with the device, but the specimen did not contain drug given the study criteria. Based on 800 specimens, the false negative rate for each device was < 1% for all drug classes. For marijuana and benzoylecgonine, and opiates all devices had ≤ 0.25% false positive rates. For PCP, the false positive rates were all ≤1.5%. For amphetamine, the false positive rates were all ≤1.75%. These rates were adjusted because study confirmation batteries included MDA, MDMA, additional OTC sympathomimetic amines, hydromorphone and hydrocodone. Without this additional testing, false positive rates approached 4% (Triage®) for amphetamines and were ≥2.25% for opiates. Fifty to 90% of the device positive amphetamine(s) samples contained MDMA. A similar percent of the opiate positive samples contained hydromorphone or hydrocodone. When additional drugs were included in the confirmation testing, it was concluded that POC urinalysis drug testing results were useful in DUI investigations.

Key Words: Point-of Care-Drug Tests, DUI, Urinalysis.

THCV as a Marker for the Ingestion of Marijuana VS Marinol®: Result of a Clinical Study.

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We have previously proposed the use of THCV (the C3 homolog of THC) as a marker for the ingestion of marijuana (or a related product) since THCV is a natural component of most cannabis products along with THC and does not exist in Marinol®. We have also reported that THCV is metabolized by human hepatocytes to THCV-COOH, therefore, the presence of the latter in a urine specimen would indicate that the donor must have used marijuana or a related product (with or without Marinol®). In this study, we provide clinical data showing that THCV-COOH is detected in urine specimens collected from human subjects only after the ingestion of marijuana and not after the ingestion of Marinol® (whether the latter is ingested orally or by smoking).

Four subjects (male and female) participated in the study in a three-session, within-subject, crossover design. The sessions were conducted at one-week intervals. Each subject received, in separate sessions and in randomized order, either an oral dose of Marinol® (15 mg), a smoked dose of Marinol® (16.88 mg) in a placebo marijuana cigarette, or a smoked dose of marijuana (2.11% THC and 0.12% THCV). Urine samples were collected and vital signs were monitored every 2 hours for a 6-hour period following drug administration. Subjects were then transported home, were given sample collection containers and logbooks, and were instructed to record at home the volume and time of every urine collection for 24 hours, and once a day for the remainder of a week (6 days). Subjects were also instructed to freeze the urine samples until the next session.

All urine samples were analyzed by GC/MS for THC-COOH and THCV-COOH using solid phase extraction and derivatization procedure on RapidTrace® and TBDMS as the derivative. The method had a limit of detection of 1.0 ng/mL and 1.0 ng/mL for THCV-COOH and THC-COOH, respectively.

Key words: Marijuana, Marinol®, THCV

Hx: analytical goal / meets clinical study — purpose of this paper
see TAT for earlier papers
elimination kinetic profile
same as THC-A

The Determination of THC-COOH in Hair using Negative Ion Chemical Ionization Gas Chromatography-Mass Spectrometry and High Volume Injection

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The determination of THC-COOH in hair specimens at the sensitivity required to detect Marijuana users, is a difficult analytical problem. To date, it has generally been accepted that triple quadropole instruments are required to achieve below 1 pg/mg concentrations in hair when two ions are monitored (either parent or daughter ions). Our method utilizes a bench-top single stage quadropole instrument adapted for high volume injection in order to achieve the required sensitivity.

Hair specimens were washed, incubated in sodium hydroxide, subjected to solid-phase extraction, Derivatized, and analyzed using high volume injection coupled with negative ion chemical ionization gas chromatography-mass spectrometry.

A common disadvantage of chemical ionization, the production of a single *m/z* ion, wasalso addressed. By specific selection of the derivatizing agent, and using ammonia as the reagent gas, three ions were monitored, allowing the calculation of two ion ratios, as in electron impact mode. Use of methane as a reagent gas, produced two abundant ions, which were used to calculate one ion ratio. The method was applied to several hair specimens taken from known marijuana users and is currently used routinely for the confirmation of workplace specimens.

A sensitive and specific method has been developed for the quantitative assay of THC-COOH in hair using high volume injection and NCI mass spectrometry.

Key Words: THC-COOH in hair; High-volume injection; Negative CI-GC/MS

Two incorporation of acidic drugs (inc THC4) into hair
Or of basic drugs
See special JAT issue for details
Key is high volume injector
Most are ≈ 1-2pg/mL

Performance Evaluation of Three On-Site Adulterant Detection Devices which Qualitatively Assess the Integrity of Urine Specimens Collected for Drugs-of-Abuse Testing

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We evaluated the performance of three on-site adulterant detection devices to assess the integrity of urine specimens collected for drugs-of-abuse testing: Intect™ 7, Mask Ultra Screen®, and Adultacheck™ 4. Intect 7 simultaneously tests creatinine, nitrite, glutaraldehyde, pH, specific gravity, and the presence of bleach and pyridinium chlorochromate (PCC). Mask Ultra Screen tests creatinine, nitrite, pH, specific gravity, and oxidants, while Adultacheck 4 tests creatinine, nitrite, glutaraldehyde, and pH. Urine specimens were prepared with the SAMSHA regulated analytes at 30% above the cut-off concentrations. Stealth™, Urine Luck™, Instant Clean ADD-IT-ive™, and KLEAR were added individually to the drug-added urine specimens such that their concentrations reflected the "optimum" usage reported in their package inserts and 25% above and below that "optimum". Stealth is reported to be peroxidase; Urine Luck is believed to be PCC; Instant Clean ADD-IT-ive reportedly contains glutaraldehyde, while KLEAR is a nitrite. The following diluents/adulterants were added at 25%, 33% and 50% the volume of drug-added urine: distilled water, bleach, ammonia, and vinegar.

Of the devices tested, Intect 7 proved to be the most sensitive device and correctly indicated the presence of adulterant or diluent in all samples tested. In order to do so, all indication pads had to be assessed in concert. Adultacheck 4 specifically assesses 4 characteristics of urine integrity and is therefore very limited in detecting the use of several popular adulterants commercially available. While it correctly assessed the 4 characteristics, it did not detect the use of Stealth, Urine Luck, or Instant Clean ADD-IT-ive. Mask Ultra Screen can potentially detect a broader range of adulterants than Adultacheck 4. However, in practice, it only detected them at levels well above their optimum usage, making it less efficacious than Intect 7. Clearly, specific identification of an adulterant is a trade-off for sensitive detection of several adulterants.

Key Words: adulterants, urine integrity, drugs-of-abuse

Intect 7 (Branan) probably best for overall screen (widest scope) but would still need tailor made specific confirmation.

P-2

Oral Fluid Drug Testing II. Evaluation of Simultaneous Oral Fluid Collections for Marijuana Testing

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Specimen collection is an important component of drug testing programs. A split specimen may be obtained by subdividing a single specimen or collecting two near-simultaneously-collected specimens. However, little information is available to validate that "near-simultaneously-collected" oral fluid specimens produce the same result. This report describes studies of healthy, male marijuana (MJ) users in which simultaneous oral fluid specimens (left and right sides of the mouth) were collected following supervised smoking of 1 MJ cigarette (Study #1, N = 10 subjects, collection times: 0-72 hrs; Study #2, N = 5 subjects, 0-1.75 hr); and the oral consumption of 1 MJ cigarette in a "Brownie" (Study #3, oral MJ, N = 3, 0-72 hrs). Each MJ cigarette contained 20-25 mg of THC. The two simultaneous specimens were collected with the Intercept™ DOA Oral Specimen Collection Device. Screening and confirmation for THC were performed with the Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) (1.0 ng/mL cutoff) and by GC-MS-MS (0.5 ng/mL cutoff), respectively. Differences were evaluated for the left- and right-sided collections. For EIA, 165 of 169 results were in agreement (97.6%; 99 positives and 66 negatives). Two specimens displayed negative results on the left side and positive results on the right side, and two specimens displayed the opposite pattern. By GC-MS-MS, 162 of 169 results were in qualitative (positive/negative) agreement (95.9%; 93 positives and 69 negatives). The 7 discordant results were unevenly divided between the left and right sides (6 -/+ results and 1 +/- results). Average THC (± SEM) concentrations for the 7 discordant specimens from the left and right sides of the oral cavity were 0.4 (± 0.1) and 0.5 (± 0.1) ng/mL. Paired t-tests indicated no significant difference ($p>0.05$). These results indicated that two oral fluid specimens, near-simultaneously collected, by two Intercept™ Collection Devices produced the same result and would meet criteria to serve as a "split specimen" as defined in the proposed DHHS guidelines for Federal Workplace Drug Testing Programs.

Key Words: Marijuana, Oral Fluids, Split Specimens

Interaction between Carbon Monoxide (CO) and Ethanol in Fire Fatalities

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Although impairment due to ethanol is clearly a risk factor in deaths due to fire, it is less clear whether or not there is a physiological interaction between ethanol and CO that would alter the carboxyhemoglobin saturation level (COHb sat.) that would account for death. In an attempt to explore this issue further, 196 fire fatalities investigated by the Office of the Chief Medical Examiner, State of Maryland over a three-year period were examined. COHb sat. and blood ethanol concentrations (BAC) were tabulated. Twelve cases positive for therapeutic or abused drugs other than lidocaine or atropine were excluded; 184 cases were included:

BAC (mg/dL)	COHb Saturation Level (%)					
	<10	10--19	20-29	30-39	40-49	> 50
<0.01	17	17	14	16	13	52
0.01-0.09	2	2	2	2	0	4
0.10-0.19	3	0	2	1	3	11
0.20-0.29	1	1	1	3	3	7
>0.30	1	0	0	3	2	1

If there is an interaction between ethanol and CO, it would be expected that as the COHb sat. increases, there would be a change in the percentage of cases positive for ethanol. Therefore, the data was reorganized to determine whether or not this in fact occurs.

COHb (% sat.)	% Cases Positive for Ethanol	COHb (%sat.)	% Cases Positive for Ethanol
<10	30	30-39	34
10--19	15	40-49	38
20-29	27	>50	31

From this table, it appears that ethanol does not affect the COHb saturation level that accounts for death, since the percentage of cases positive for ethanol at a given COHb range shows no trends. Therefore, we conclude that although ethanol remains a risk factor in fire fatalities, the risk appears to be related to the impairment that it produces as opposed to a direct interaction with CO.

Key Words: carbon monoxide, ethanol, interaction

P-4

Oral Fluid Drug Testing III. Comparison of Oral Fluid Testing with Urine Testing for Marijuana

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Detection of very recent drug use is an important advantage of oral fluid over other specimens in workplace and safety-sensitive drug testing programs. This report describes a study with healthy, male marijuana (MJ) users in which oral fluid specimens were collected simultaneously with timed urine specimens following supervised smoking of 1 MJ cigarette (N = 10 subjects, collection times: 0-72 hrs). Each MJ cigarette contained 20-25 mg of THC. Oral specimens were collected with the Intercept™ DOA Oral Specimen Collection Device. Specimens were screened for THC with the Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) (1.0 ng/mL cutoff) and confirmed for THC by GC-MS-MS (0.5 ng/mL cutoff). Urine specimens were screened for MJ metabolites by EIA (50 ng/mL cutoff) and confirmed for 9-carboxy-THC by GC-MS (15 ng/mL cutoff). All oral fluid specimens initially tested negative by EIA and GC-MS-MS for THC prior to smoking MJ. At 1 hr after smoking MJ, all oral fluid specimens tested positive (100%) for THC by both methods. Positive rates were 90% through 4 hrs, 60-80% at 16 hrs, 10-20% at 24 hrs, and negative at 48 hrs; however, at 72 hrs three positive specimens (30%) were detected by EIA and confirmed by GC-MS-MS. Positive rates by urine testing were generally lower than by oral fluid testing over the first 16 hrs. The average positive rate for urine specimens at 1 hr was 22% by EIA. Thereafter, positive rates by EIA rose slowly to a peak positive rate of 70% at 16 hrs and then declined to 30% at 24 hrs. EIA positive rates at 48 hrs and 72 hrs were 20% and 40%, respectively. The average positive rate for 9-carboxy-THC by GC-MS was 11% at 1 hr. The positive rate rose to 50% at 2 hrs and was 70% at 4 hrs; the rate remained in this range through 48 hrs and declined to 50% at 72 hrs. Overall, there was a substantially higher rate of detection over the first eight hours of positive specimens by oral fluid testing compared to urine testing as a result of a "lag" period between the time of MJ use and the time required for THC absorption, distribution, metabolism, and excretion in urine to occur.

Key Words: Marijuana, Oral Fluid, Urine

P-5

Post-Mortem Distribution of Heroin Metabolites in Femoral Blood, Liver, Cerebral Spinal Fluid, and Vitreous Humor.

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In humans, heroin (diacetylmorphine) is subject to rapid metabolism to 6-monoacetylmorphine (6-MAM) and then to morphine. The presence of 6-MAM is often used to distinguish between heroin and morphine overdose. This metabolite, however, is rapidly converted to morphine and may not be present in detectable quantities following heroin exposure. Recent studies (Jenkins and Lavins, 1998, *JAT, 22:173*) have shown that 6-MAM may persist in cerebral spinal fluid (CSF) and this is a preferable specimen for establishing heroin overdose. Codeine is a common contaminant of heroin and is often found in cases of heroin overdose. This study reports the post-mortem distribution of heroin metabolites (6-MAM, unconjugated morphine, and codeine) in femoral blood, liver, CSF, and vitreous humor (VH) in twenty-five deceased individuals. In all cases, 6-MAM was detected in VH, and in CSF in 16 of the 25 cases (64 %). For those 16 cases where 6-MAM was detected in both VH and CSF, the concentration of 6-MAM was higher in VH 75 % (12/16) of the time. When 6-MAM was detected in blood, (13 of 25 cases) it was also found in VH and CSF; the level of 6-MAM in VH and CSF was higher than in blood, with a mean concentration ratio of 11.3 (range:1.7 to 27) for VH and 6.6 (range: 2.6 to 17.3) for CSF. 6-MAM was not present in liver in any of the cases examined. Free morphine levels were highest in liver, followed by blood, CSF and VH. The concentration ratios (mean ± S.D.) for free morphine in VH, CSF and liver to that in blood were 0.36 ± 0.18, 0.64 ± 0.27, and 2.99 ± 2.12, respectively. The liver:blood ratio was consistent with previously reported values for morphine in heart and femoral blood. Codeine levels following heroin overdose were consistently low relative to the morphine concentration. For blood, VH, and CSF, the ratio of codeine to blood morphine was essentially the same (0.06), while liver codeine concentration was slightly higher (0.16). These results characterize the distribution of heroin metabolites in post-mortem tissues. VH appears to be the most useful specimen for determining 6-MAM, and establishing that heroin was the source of morphine.

KEY WORDS: 6-Monoacetylmorphine, post-mortem, vitreous humor

P-6

Benzphetamine; A Case Report

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Abuse of Benzphetamine, a sympathomimetic amine used in the treatment of obesity, has recently increased in cities of southwest Ohio. A total of two solid dose drug cases received in the crime laboratory were identified as Benzphetamine in the year 2000, compared to a total of six cases between January and May in 2001. The toxicological data for this drug is not well characterized with regard to parent drug distribution, metobolite recovery, and analytical methods. The major metabolites of Benzphetamine are Methamphetamine and Amphetamine. Pharmacologically, Benzphetamine differs from Amphetamine in that it has reduced stimulant and cardiovascular effects, while maintaining its anorectic efficacy. In conjunction with increase abuse of the drug, Benzphetamine was implicated in the death of a 45-year old white male found unresponsive by his spouse. In this case, there was a history of drug abuse and the spouse provided an unmarked bottle containing five Didrex (50mg) tablets that the decedent bought from "street sources". Significant autopsy findings included a heart weight of 715 grams with moderate interstitial fibrosis and endocardial fibrosis. The concentration of Benzphetamine was measured in heart blood, urine, bile, gastric fluid, vitreous humor, cerebral spinal fluid, brain, and liver. The analysis was performed using liquid-liquid extraction, followed by derivatizing samples with BSTFA+1%TMCS, and injecting on GC-MS. Quantitative results for Benzphetamine were 0.26, 0.172, 0.135, 0.169, 0.048ug/mL for urine, brain, liver, gastric fluid, and bile, respectively. No parent drug was detected in the blood. Methamphetamine concentrations were 1.21, 0.011, 0.04, 0.03ug/mL for urine, vitreous humor, brain, and bile, repectively, while Amphetamine concentrations were 0.041, 14.44, 0.147, 0.496, 0.48, 0.918, 0.311, 0.056ug/mL for femoral blood, urine, vitreous humor, brain, liver, gastric fluid, bile, and cerebral spinal fluid, respectively. The cause of death was ruled as cardiac arrhythmia, due to cardiomegaly, with amphetamine use contributing.

Key Words: Benzphetamine, Toxicity, Tissue Distribution

P-7

Either "Urine Luck" or Not: Determination of Chromate Adulteration of Human Urine by Colorimetric and Capillary Ion Electrophoretic Analyses.

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Various chemicals can be added to urine specimens collected from drug analysis to abnormally elevate ionic concentrations and/or interfere with either immunoassay urine drug screening procedures or gas chromatographic/ mass spectrometric confirmation techniques. One such adulterant, "Urine Luck" has been identified from our previous research to contain potassium dichromate. Screening of suspected adulterated specimens and confirma-tion of the adulterant is important for forensic drug screening. Development and application of colorimetric and capillary ion electrophoresis (CIE) techniques for the detection and confirmation of chromate adulteration of urine specimens was the purpose of this investigation. Thirty six urine specimens suspected of adulteration were analyzed for chromate by colorimetric analysis with diphenylcarbazide on a Wako 30R random access automated chemistry analyzer (Behring Diagnostics, San Jose, CA). Chromate in urine complexes with diphenylcarbazide to form a colored chromophore which can be quantitated by measurement of absorbance at 540 nm. Duplicate aliqouts were analyzed for chromate by CIE on a Waters Quanta 4000 Capillary Electrophoresis System using an uncoated 75 μm (i.d.) X 375 μm (o.d.) X 60 cm (length) capillary with a voltage of 18 kV, a current of 50 μamps, and direct ultraviolet absorption detection at 254 nm. Chromate was detected in aliquots of urine diluted 1 to 10 with deionized water, vortexed and analyzed using a run electrolyte of 12.5 mM monosodium phosphate/ 12.5 mM disodium phosphate/ 3.5 mM tetradecyltrimethylammonium hydroxide (OFM-OH) at pH of 7.0. Chromate was eluted and detected at 3.12 minutes. Results of the colorimetric chromate analyses revealed a mean chromate concentration of 929 μg/ml with a standard error of 177 μg/ml and a range of 30 to 5634 μg/ml. Results of the CIE chromate analyses revealed a mean chromate concentration of 1009 μg/ml with a standard error of 218 μg/ml and a range of 20 to 7501 μg/ml. The correlation coefficient between the CIE and colorimetric chromate results was $r = 0.9669$. Application of the diphenylcarbazide colorimetric technique provides rapid determination of chromate adulteration of a urine specimen. CIE offers a separation technique to confirm the presence of chromate in suspected adulterated specimens. The excellent correlation between these methods substantiates their application to forensic testing as screening and/or confirmation techniques.

Key Words: Capillary ion electrophoresis, chromate, urine adulterants.

P-8

Site Study: Determination of Abused Drugs and Adulterants at Various Testing Facilities by the Scooper™ Test Cup

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The Scooper™ test cup integrates a sample container for transportation with a test device that detects 5 abused drugs -- THC, benzoyl ecgonine, morphine, amphetamine, and PCP. It also simultaneously detects 4 adulterant parameters -- oxidizing agents, pH, creatinine and nitrite. To run the test, urine sample is first collected in a cup base and then a test lid is attached. Test reactions are initiated by a one-step action of pushing and releasing a lever on the test lid. The presence or absence of an adulterant is achieved by comparing the color of the specific adulterant line with that of a given color chart. In the drug test panel, the presence of a red line in the region corresponding to a specific drug indicates the absence of such a drug in the sample.

Sixty urine samples each containing five abused drugs at one of these concentrations (negative, 50% below cut-off, cut-off, 50% above cut-off and three times cut-off) were tested with the Scooper cup over 3 days at three sites: 1) a toxicology laboratory; 2) a treatment center; 3) a nursing station in a large manufacturing plant. The procedure was designed to be a blind study and was carried out by the site personnel after a brief training period. Urine samples containing abused drugs above the cut-off levels should produce a positive result while samples with cut-off of lesser drug concentrations should produce a negative result. Reported data from all three sites indicated 100% correct determination of positive and negative results.

Four sets of 6 drug free urine samples were then prepared. Each set was spiked with one of the following adulterants at the stated concentrations: 1) bleach (at 5% and 10% by volume); 2) sodium nitrite (at 1.5 mg/dL and 13.5 mg/dL); 3) pH (acidic buffer at 2.5 and alkaline buffer at 9.5); and 4) creatinine (at 11 mg/dL and 95 mg/dL). These urine samples were tested blind with the Scooper cup at the toxicology laboratory and 100% correct results were reported.

The present study suggests that the Scooper test cup can provide testing of adulterants and abused drugs accurately in various testing facilities.

Keywords: Abused Drug, Adulterant, Workplace Drug Testing

P-9

Status of Drug Absorption in Hair of Chronic Drug Abusers from Autopsy Specimens

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Hair from autopsies of drug overdose cases of chronic drug users was analyzed in a segmental fashion. The hair was washed, digested/extracted and injected into an ion trap gas chromatograph/mass spectrometer for analysis. When available, root bulbs were also analyzed. The root bulbs give an indication of the amount of drug in the body at the time of death. The drug overdose cases were either from cocaine, opiate, and/or amphetamine users. From the 29 cocaine/benzoylecgonine positive hair specimens, the amounts of drug ranged from 0.91-158.5 ng/mg for cocaine and 0.11-50.4 ng/mg for benzoylecgonine. From the available root bulbs, 19 specimens ranged from 0.6 – 171.5 ng/mg of cocaine and 11 specimens ranged from 2.7 – 15.4 ng/mg of benzoylecgonine. From the 20, 6-acetyl morphine/morphine positive hair specimens, the amounts of drug ranged from 0.06 – 25.4 ng/mg for 6-acetylmorphine and 0.01-11.6 ng/mg for morphine. From the available root bulbs, 3 specimens ranged from 1.47 –15.11 ng/mg of 6-acetyl morphine and 4 specimens ranged from 0.06 – 8.67 ng/mg of morphine. From the 10 methamphetamine/amphetamine positive hair specimens, the amounts of drug ranged from 0.25 – 323.6 ng/mg for methamphetamine and 0.1- 36.68 ng/mg for amphetamine. From the available root bulbs, 8 specimens ranged from 0.54 –51420.3 ng/mg of methamphetamine and 8 specimens ranged from 0.3 – 41.2 ng/mg of amphetamine. These data indicate the typical amounts of drugs to be expected in hair from chronic drug users. The root bulb data indicate that a rapid uptake of drug is experienced in the root bulbs, and depending on the chemical properties of the drug, the drug is either primarily incorporated into the hair shaft upon keratization or primarily expelled upon keratization.

Key Words: Hair, Drug Absorption, Chronic Users

P-10

ADDICTED TO DUI – A GHB/GBL CASE REPORT

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We present a case report of a 38 year-old male who was arrested 7 times over an 8-month period for driving under the influence of drugs. In each incident, GHB was determined to be the causative agent.

A blood specimen was drawn between 1.5 to 2.5 hours after first police contact in each occasion. GHB was analyzed by GC-MS, following extraction from blood using ethyl acetate and subsequent derivatization using BSTFA/TMCS. Blood specimens additionally underwent alcohol analysis, an EMIT screen for drugs of abuse and several prescription drug classes, and a GCMS screen for acidic, neutral and basic compounds.

Blood GHB concentrations ranged from 44 to 184 mg/L (mean 100 mg/L, median 73 mg/L). Signs of impairment generally included erratic driving (severe lane travel, collisions and near-collisions), slurred speech, disorientation, slow to react, shaking, agitation, unable to focus, poor coordination and balance, poor performance in field sobriety tests, somnolence, and unconsciousness. On only one occasion, other drugs were present in the blood, namely thiopental 8.7 mg/L and diazepam 0.08 mg/L, which may have contributed to the observed driving impairment.

During several police interviews, the subject stated he was addicted to GHB and GBL, and had admitted taken "RenewTrient", "Dream On", "V35", "fitness supplements", or "GBL". He also stated he was taking Paxil® (paroxetine) and Adderall® (amphetamine) for depression, however, neither drug was detected in any of the blood specimens. During the same period, the subject had been admitted numerous times to several hospitals for GHB/GBL intoxications.

Keywords: GHB, Driving Impairment, Addiction

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P-11

Development of Monitect™ MDMA – an Onsite Ecstasy Drug Screen

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We are in the process of completing the development of Monitect MDMA -- an onsite Ecstasy drug screen and would like to present our preliminary data as follows.

The current test utilizes the lateral flow technology with MDMA monoclonal antibody conjugated to colloidal gold particles to generate a semi-quantitative standard curve with the cut-off concentration tentatively set at 1000 ng/ml of MDMA. Urine samples spiked with MDMA over 1250 ng/ml all show positive results while samples with 1000 ng/ml or less of MDMA show negative.

With MDA, over 2000 ng/ml was required to produce a positive result. Other critical cross-reactants like amphetamine, methamphetamine, ephedrine, pseudoephedrine and phenylpropanolamine all show very low cross-reactivity requiring at least 100 ug/ml concentration to produce a positive result.

100 known negative urine samples were all tested negatives with the Monitect MDMA. Preliminary clinical sample study shows the Monitect MDMA result correlates well with GC/MS data.

These results suggest that the Monitect MDMA should be a viable onsite Ecstasy drug screen.

Keywords: Ecstasy, MDMA, Onsite Drug Screen

P-12

Analysis of Gama-Hydroxybutyrate (GHB) in Human Plasma by Gas Chromatography - Positive Chemical Ionization Mass Spectrometry

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Gamma-hydroxybutyrate (GHB) is a metabolite of the neurotransmitter gamma-aminobutyric acid. GHB has anesthetic and hypnotic properties and its use as a drug of abuse and a date rape drug has increased in recent years. Following the administration of GHB in doses that produce drowsiness, peak GHB concentration were reported to average 80 µg/mL (1). We have developed an efficient method to measure GHB in plasma samples by GC-MS. The internal standard used was GHB-d_6. The method involves protein precipitation and extraction with acetonitrile and derivatization with BSTFA (+ 1% TMCS). The extracts were analyzed with an Agilent 5973 GC-MSD instrument and a (5% -Phenyl)-methylpolysiloxane capillary column. The extracts were analyzed using positive chemical ionization (PCI). Ammonia was used as the reagent gas and the ion source temperature was 200° C. Under these conditions, the TMS derivatives of GHB and GHB-d_6 had mass spectrums with prominent protonated molecular ions at (m/z) 249 and (m/z) 255. The analysis was linear from 1-250 µg/mL. The precision and accuracy of the analysis was evaluated at 5, 75, and 150 µg/mL GHB. The coefficient of variation was less than 7% for the intra-assay precision. In the accuracy evaluation, the GHB concentrations were within 3% of target. The procedure shows that PCI is an effective means of ionization for analyzing GHB by GC-MS.

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Key words: Gama-hydroxybutyrate (GHB), GC-MS, Positive Chemical Ionization (PCI)

1. P. Palatini, L. Tedeschi, G. Frison et al. Dose-dependent absorbtion and elimination of gamma-hydroxybutryric acid in healthy volunteers. Eur. J. Clinical Pharm. 45:353-356, 1993.

0.1 ml sample
0.05 d_6 GHB (10ng/mL)
2 mL ACN
V+C

Sel rspmt

P-13

The Modern Party: An Insider's look at Raves in the Phoenix Metropolitan Area

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Rave music and its culture were born in the mid 1980's as music moved from disco to techno. PLUR (Peace, Love, Unity, Respect) was one of the initial catch phrases. Raves were initially about the music and freedom from legal and social rules. For many Ravers the Rave is almost a spiritual experience often aided by drugs.

The abundance of Raves in Arizona was brought to light the past several years beginning with the arrest of Sammy "The Bull" Gravano's Ecstasy ring. Shortly after Dateline NBC did a special report on Raves, following a teenage girl at several parties in Arizona.

This presentation will take a look at Raves from several points of view including the Promoter, the Raver, and Law Enforcement. It will also help educate about Raves and the Rave Culture from the presenters personal experiences and observations. The presentation will also look at "club drug" submissions versus toxicology findings statistics for Arizona and offer possible explanations.

Keywords: Rave Culture, Ecstasy, Club Drugs

P-14

GC/MS Ketamine Analysis of Urine Samples Positive for MDMA in U.S. Navy and Marine Corps Personnel

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Ecstasy (3, 4-methylenedioxymethamphetamine, MDMA), designer drugs (compounds with substitutional variations to amphetamine), and other hallucinogens like LSD (lysergic acid diethylamide) and Ketamine have been associated with all night dance clubs called Rave parties. The popularity of the "Rave" scene with teenagers and young adults and the ease of availability of these illicit drugs to military service members who attend these parties have become a serious concern for the U.S. Department of Defense (DoD). Despite the DoD's drug screening program and its inclusion of screening for the presence of MDMA and LSD, presently Ketamine is not screened due to the lack of a commercially available immunoassay kit. Recent reports have revealed that there has been a significant increase in the use of Ketamine as a club drug (Drug Detection Report, 11, 5, March 8, 2001). There have also been reports of confiscated tablets containing mixtures of Ketamine and MDMA (Microgram, 37, 1, January 2001).

The Laboratory has observed a 1,450 % increase in the number of MDMA positive samples between the years of 1997 (42) and 2000 (609). The potential for a significant increase in the number of Ketamine positive urine samples from the same population group should mirror that of Ecstasy. Therefore, the objective of this study was to investigate the prevalence of Ketamine in 200 U.S. Navy and Marine Corps Service member urine samples that were previously confirmed positive for Ecstasy ($\geq$ 500 ng/ml).

Key Words: Ketamine, Ecstasy, and GC/MS

P-15

Fatalities Caused by the MDMA-Related Drug, *Para*-Methoxyamphetamine (PMA)

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The past several years have seen a marked increase in the recreational use of 3,4-methylenedioxymethamphetamine (MDMA) or "Ecstasy". MDMA use is especially common among young people participating in dance parties called "raves". Para-methoxyamphetamine (PMA) exhibits both structural and pharmacological similarity to MDMA. It may, however, be a more potent central stimulant, particularly in its effects on serotonergic transmission.

While several fatalities from PMA have been reported in Australia, here we report three recent fatalities that have occurred in the Midwestern United States in which each of the decedents believed that they were ingesting MDMA. Symptoms observed included agitation and bruxism progressing to severe hyperthermia, convulsions and hemorrhage. Blood was screened for drugs of abuse by enzyme immunoassay with the presence of amphetamines indicated in each case. PMA was also identified in GC/MS alkaline blood screens. Confirmation and quantitation for amphetamines was performed by GC/MS. The deceased, two males ages 19 and 24 and a female age 18, had postmortem blood PMA concentrations of 1.07, 0.60, and 1.90 mg/L, respectively. PMA is not a contaminant of MDMA and no MDMA was found in any of these cases. The primary metabolite of PMA is produced by O-demethylation to 4-hydroxyamphetamine, a reaction catalyzed by cytochrome P450 2D6. This enzyme is noted to be genetically polymorphic. Those with the "slow metabolizer" phenotype may be likely to have higher peak blood concentrations of PMA. Whether any of the decedents described herein were of the "slow metabolizer" phenotype is not known.

Several groups have advocated the on-site use of the Marquis Test for the purpose of pill screening in efforts to distinguish PMA from MDMA. A dark purple is consistent with MDMA whereas PMA imparts no color change in this test. PMA is often in the form of a white pill with a Mitsubishi symbol on one side. This design has been identified in at least one of these fatalities. While MDMA use is frequent among those at dance parties, in each of the fatalities presented here the decedent used the drug they believed to be MDMA in their home or that of a friend.

Key Words: *Para*-Methoxyamphetamine, PMA, MDMA

Oral Fluid Drug Testing I. Timecourse of Δ-9-Tetrahydrocannabinol (THC) in the Oral Cavity After Smoked and Oral Marijuana

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Detection of THC in oral fluid specimens provides a convenient, non-invasive method of testing for marijuana (MJ). However, its time course of detection has not been well characterized. In studies conducted with healthy, male MJ users, we evaluated the time course of THC in oral fluid following supervised smoking of 1 MJ cigarette (Study #1, N = 10 subjects, collection times: 0-72 hrs; Study #2, N = 5 subjects, 0-1.75 hr); and the oral consumption of 1 MJ cigarette in a "Brownie" (Study #3, oral MJ, N =3, 0-72 hrs). Each MJ cigarette contained 20-25 mg of THC. Simultaneous oral fluid specimens were collected from the left and right sides of the mouth with the Intercept™ DOA Oral Specimen Collection Device. Specimens were screened with the Cannabinoids Intercept™ MICRO-PLATE Enzyme Immunoassay (EIA) (1.0 ng/mL cutoff) and confirmed for THC by GC-MS-MS (0.5 ng/mL cutoff). Highest THC concentrations (Cmax) were in the first oral fluid specimen. Mean Cmax ± SEM (ng/mL) and range by GC-MS-MS were as follows: Study #1, left, 25.5 ± 4.8, 2-45; Study #1, right, 24.0 ± 4.3, 2-44; Study #2, left, 80.6 ± 37.7, 15-228.2; Study #2, right, 59.4 ± 21.6, 8.6-136.4; Study #3, left, 3.4 ± 1.9, 1.2-6.9; Study #3, right, 4.8 ± 2.8, 2.2-7.1. THC concentrations declined rapidly over 2-4 hours but remained detectable up to 72 hrs (4 of 10 subjects). Mean detection times (hr) and range for THC in left-side specimens were as follows: Study #1, last consecutive positive, EIA 14.5 ± 1.9, 1-24, GC-MS-MS 12.9 ± 2.6, 1-24; Study #1, last positive, EIA 30.5 ± 9.2, 1-72, GC-MS-MS 33.7 ± 10.5, 1-72; Study #3, last consecutive positive, EIA 26.7 ± 22.7, 4-72, GC-MS-MS 7.3 ± 4.4, 2-16; Study #3, last positive, EIA 26.7 ± 22.7, 4-72, GC-MS-MS 26.0 ± 23.0, 2-72. It was concluded that although a sensitive methodology is necessary, oral fluid can be a reliable specimen for detection of recent MJ use.

Key Words: Marijuana, Oral Fluids, Detection

$[\,]_{oral} \approx$ v. similar to $[\,]_{plasma}$ ~~t_{max}~~ THC not THCA

$\approx 25 ng/mL \times 3 = 75 ng/mL$ @ 1 hour

$[\,]_{oral} << [\,]_{smoking}$

detection time $\approx$ 1 day

t_{max} 15 min

Application of a Convenient Procedure to Analyze GHB for Fatalaties involving Gammahydroxybutyric acid, Gammabutyrolactone and 1,4-Butanediol.

*By WC Duer, KL Byers*and JV Martin*

The most common chemicals which can be ingested and lead to greater than endogenous levels of gammahydroxybutyrate, GHB, in decedents are salts of GHB; GBL; and, 1,4-butanediol, BD. Results for three cases are presented for deaths involving the ingestion of one or another of these three chemicals which led to findings of GHB in the decedents. An extraction procedure was developed which facilitates the quantitation of GHB. GBL can be determined using the procedure after converting GBL to GHB. If present in the same specimen, both GHB and GBL can be quantified. The extracts were quantitated by GC/MS as the di (trimethylsilyl) derivative of GHB. Analyses were made using either hexadeuteroGHB or gammahydroxyvalarate, GVA, as the internal standard. For quantitative purposes, these two internal standards were demonstrated to be equivalent in terms of limit of detection, limit of quantitation and linear dynamic range A separate procedure was developed for estimating concentrations of BD by using high performance liquid chromatography with a derivative formed from BD and 3,4-dinitrobenzoyl chloride. Specimens analyzed included urine, blood, ocular fluid, brain and solutions consumed by the decedents prior to death. The decedents ingested either solutions of GHB, GBL or BD. In the case where GHB was ingested, the GHB had been added to a commercial sports drink to a concentration of 125,000 mg/L. In the case where GBL was ingested, the GBL was present in a liquid product sold for presumed health benefits and was found to contain 19,000 mg of GBL/L. In the case where BD was ingested, the BD was in both pure form and as an aqueous solution with a concentration of 50,000 mg/L.

Key Words: Gammahydroxybutyrate, Gammabutyrolactone, 1,4-Butanediol.

Pharmacogenomics for Forensic Pathology-Toxicology: Genotyping CYP 450 2D6 as an adjunct for certifying antidepressant toxicity

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Pharmacogenomics(PG) may serve as an adjunct in identifying drug toxicity for the purpose of certifying the cause and manner of deaths. PG is a well established scientific and an emerging clinical discipline which correlates drug dosage/concentrations and response to genomics of the patient drug metabolizing enzyme and receptors. By stratifying patient's genotype in clinical trials and in routine patient therapy, a rational drug regimen may be designed for optimal therapeutic response and minimized drug toxicity, greatly enhancing the drug discovery process and routine therapy. One group of the drug metabolizing enzymes, CYP 450 2D6 is regulated by genes in chromosome 22. In particular, the gene CYP 450 2D6 is polymorphic. Depending on mutation, patients may demonstrate phenotype of a poor, normal or rapid drug metabolizer. Genotyping drug metabolizing enzymes is currently regarded as one of the enabling technologies for ' Made to order ' drug therapy - medicine. This preliminary study is concerned with the application of PG as an adjunct for certifying antidepressant toxicity. The decedent was a 43 year old female with obsessive compulsive disorder and asthma. Prior to her death, she informed her mother of being ill - gastrointestinal complaints including diarrhea and vomiting. She collapsed on the kitchen floor. No trauma was noted at the scene. No previous suicide attempt was reported. Autopsy findings were unremarkable. Toxicology analysis showed a blood concentration of 1.48 mg/L of clomipramine, and non-detectable nor-clompramine. Genotyping was directed for CYP 450 2D6*3, *4 and *5. After DNA extraction of whole blood, PCR analysis was performed by using recently published procedures of either conventional and/or real time PCR for 2D6*3 and *4, and long PCR for 2D6*5. The results showed the decedent to be 2D6*4 heterozygous. The current interpretation suggests 2D6*4 heterozygosity correlates up to 80% of the normal drug metabolism. The cause of death was certified to be clomipramine toxicity with CYP450 2D6*4 heterozygosity, and manner of death – accidental.

Key words: Pharmacogenomics, CYP 450 2D6, and clomipramine.

A Case of Homicide: Chronic Ethylene Glycol Poisoning.

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We present a homicide due to chronic ethylene glycol poisoning. The victim was 55 year old housewife with a long history of manic depression treated with lithium, fluphenazine and conjugated estrogens. Shortly after marrying her second husband, she developed a yearlong chronic illness characterized by recurrent bouts of nausea, vomiting, lethargy, mental confusion, metabolic acidosis and chronic renal failure that resulted in twenty-eight hospitalizations. This illness was continuously diagnosed as a possible overdose of her medications, although, lithium serum values were always within therapeutic concentrations. Two weeks prior to her death, she presented at an emergency department with an apparent psychotic episode and metabolic acidosis. A serum ethylene glycol test revealed a concentration of 6 mg/dL which was considered "non-toxic". Eventually, she was found at home unresponsive and brain dead. At autopsy, crystals consistent with calcium oxalate were present in the renal tubules and perivascular spaces of the brain. Postmortem blood contained 94 mmol/L oxalic acid. The cause of death was attributed to ethylene glycol poisoning.

After a two-year investigation, the victim's husband admitted continuously adding small amounts of ethylene glycol to her Gatorade. He pleaded guilty to first-degree manslaughter. This case exemplifies the difficulty of diagnosing chronic poisoning, particularly in the presence of a confounding medical history and clinical presentation.

Key Words: Ethylene glycol, homicide, metabolic acidosis

Comparison of TLC vs. GC/MS Results for Urine Samples that Test Positive for Amphetamines by Immunoassay

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Does a positive immunoassay for "amphetamines" provide a reliable result without confirmation? Can TLC provide a confirmatory result for amphetamine and/or methamphetamine comparable to GC/MS?

Aliquots of 415 urine specimens that tested positive by an immunoassay for "amphetamines" were obtained from three federally or state approved laboratories. These cooperating laboratories also analyzed each sample for amphetamine and/or methamphetamine by GC/MS and provided their results to a referee. Each sample was analyzed by TLC and the results were reported to the referee. Comparison of the TLC analyses with the GC/MS data will be presented.

Results indicate that TLC was able to correctly identify those specimens found to contain amphetamine and/or methamphetamine by GC/MS. In many specimens other substances were detected by TLC. These included sympathomimetic amines, MDA, MDMA and some non-sympathomimetic amines such as ranitidine, phenothiazines and diphenhydramine.

This study demonstrates that: 1) TLC is comparable to GC/MS for the determination of amphetamine and/or methamphetamine, and 2) immunoassays for amphetamines must be confirmed to determine if the usage of a proscribed drug is in question.

Key Words: Amphetamine assays, TLC, GC/MS

Use, Misuse, and Abuse of Ephedrine & Pseudoephedrine

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Ephedrine is the parent alkaloid occurring in many of the *Ephedra* species. Ephedrine is used in asthma medications for its bronchodilation effects. Ephedrine is also a component of many herbal remedies that are marketed to promote weight loss, give a heightened sense of energy, and to promote bodybuilding. Pseudoephedrine is a structurally similar alkaloid compound also found in many plants *Ephedra* species. It is a component of many over-the-counter cough, cold, and allergy medications primarily for its decongestant properties.

Due to the high popularity of these compounds and controversy surrounding them, the concentration of ephedrine and pseudoephedrine in tissues and their contribution to the cause of death for the past three years was investigated. Cases in which ephedrine and/or pseudoephedrine were detected in postmortem blood were identified, and then the complete autopsy file in each case was reviewed. Of 797 overdose cases, seven deaths in which ephedrine and/or pseudoephedrine were detected in postmortem blood were identified. Ephedrine was detected in four of those cases, and pseudoephedrine was detected in six of the cases. The cause of death in six cases was multiple drug toxicity and the cause of death in one case was complications of acquired immune deficiency syndrome (AIDS). The manner of death was determined to be accidental in five cases, suicide in one case, and natural in one case. The following Table indicates the concentration of ephedrine and pseudoephedrine in blood for each case.

Case	Ephedrine	Pseudoephedrine
1	None detected	0.1 mg/L
2	Less than 0.1 mg/L	0.33 mg/L
3	0.12 mg/L	None detected
4	0.1 mg/L	0.12 mg/L
5	None detected	Less than 0.1 mg/L
6	0.3 mg/L	7.1 mg/L
7	None detected	0.22 mg/L

Of the seven deaths in which ephedrine and/or pseudoephedrine were detected in postmortem blood, none of the decedents had a history of taking ephedrine-containing dietary supplements. The concentrations of ephedrine and/or pseudoephedrine detected in postmortem blood were not sufficiently elevated to cause toxicity or death, with the exception of one case in which the pseudoephedrine concentration in blood was 7.1 mg/L. In conclusion that pseudoephedrine overdose contributed to one fatality in Harris County from 1997 to 2000 and ephedrine was not a contributing factor in any fatalities during that period.

Key Words: Ephedrine, Pseudoephedrine, and Harris County Medical Examiner Office.

Solid phase extraction and GC/MS analysis of THC-COOH method optimized for a high throughput forensic drug-testing laboratory.

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To facilitate the confirmation analysis of large numbers of urine samples previously screened positive for THC, an extraction, derivitization and GC/MS analysis method was developed. This method utilized a positive pressure manifold anion-exchange polymer-based solid phase extraction followed by elution directly into the automated liquid sampling (ALS) vials. Rapid derivitization was accomplished using pentafluoropropionic anhydride/ pentafluoropropanol (PFPA/PFPOH). Recoveries averaged 95% with a limit of detection of 0.875 ng/ml with a 3 ml sample volume. Performance of D3 and D9 11-Nor-delta-9-tetrahydrocannabinol-9-carboxylic Acid (THC-COOH) internal standards were evaluated. The method was linear to 900ng/ml THC-COOH using the D9 with negligible contribution from the internal standard to very weak samples. Excellent agreement was seen with previous quantitations of human samples. Greater than one thousand human samples were analyzed using the method with 300 samples analyzed using an alternate qualifier ion (m/z 622) after some interference was observed with a qualifier ion (m/z 489). The 622 ion did not exhibit any interference even in samples with interfering peaks present in the 489 ion. The method resulted in dramatic reductions in processing time, waste production and exposure hazards to laboratory personnel.

Key Words: THC-COOH, urinalysis, solid-phase extraction

Rapid and Sensitive Analysis of THC and COOH-THC in Whole Blood by Disposable Pipette Extraction

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Recently, a rapid extraction method has been developed that is referred to as disposable pipette extraction (DPX). DPX uses a pipette tip that is fitted with a frit, and it contains loosely packed sorbent material for performing solid-phase extraction (SPE). Sample is drawn into the tip via pipette, and after an equilibration time, the sample is delivered to waste. After a rapid wash step, adsorbed analyte is eluted with only 0.4 mL of organic solvent. Some of the advantages of DPX are that a time-consuming conditioning step and large solvent volumes are not required. DPX has been shown to be almost ten times faster than conventional SPE.

The analysis of tetrahydrocannabinol (THC) and carboxy-tetrahydrocannabinol (COOH-THC) in blood is tedious and time-consuming by conventional methods. DPX has been successfully applied to the analysis of THC and COOH-THC in whole blood. Spiked whole blood (0.2 mL) was first treated with 0.2 mL acetonitrile, and the supernatant was transferred to a clean test tube following centrifugation. Water was added to the supernatant and the sample was extracted by DPX. Only 0.4 mL of wash solvent (33% acetonitrile in water) and 0.4 mL of elution solvent (50% ethyl acetate in acetonitrile) was used for the extraction. The total extraction time (after protein precipitation) took less than 3 minutes per sample!

The extract was reconstituted with 25 uL ethyl acetate, transferred to a high recovery vial, and derivatized with 25 uL BSTFA at 90 C for 20 min. The limits of detection by GC/MS (in SIM mode) were 0.2 ng/mL and 0.6 ng/mL for THC and COOH-THC, respectively, with less than 8% RSD for both analytes.

Key Words: Disposable Pipette Extraction, Solid-Phase Extraction and GC/MS

Add SIMU - THC, 11-OH, THCA
single GC/MS run - details to be sent
~#2/cartridge - ACN ppt of blood, ~~then~~
then automated. Uses handheld device
allows for ~~4 samples~~ 4 samples/set

Determination of blood cyanide by HPLC/MS after simultaneous microdiffusion and derivatization by taurine and naphthalene-2,3-dicarboxaldehyde

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An original procedure involving HPLC coupled to mass spectrometry (HPLC/MS) was developed for the determination of cyanide (CN) in whole blood.

To 2 ml of blood in a 20-ml headspace vial used as a microdiffusion chamber were added 50 µl of a 1.43 mM internal standard solution of $K^{13}C^{15}N$ (Aldrich, Ref 49,053-9). A 1.5-ml Eppendorff-type microtube was then inserted into this vial (to serve as the inner chamber of the microdiffusion system) and filled with 40 µl of taurine (50 mM in deionized water)/naphthalene-2,3-dicarboxaldehyde (NDA, 10 mM in methanol)/methanol/concentrated (*ca.* 20 %) ammonia solution (25 : 25 : 45 : 5, v/v). Concentrated H_2SO_4 was added to the blood sample, then the headspace vial was sealed. After 30-min gentle agitation the contents of the inner vial were pipetted and 2 µl of this mixture were directly injected onto the HPLC column. Separation was performed on a NovaPak C18 (Waters) column (150 x 2.0 mm, i.d.), with a gradient of acetonitrile in 2 mM NH_4COOH, pH 3.0 buffer (35-80 % in 10 min). Detection was done by a Perkin-Elmer Sciex API-100 mass analyzer with an ionspray interface, operated in the negative ionization mode. MS data were collected as either TIC or SIM at *m/z* {299 + 191} and {301 + 193} for the derivatives formed with CN and $^{13}C^{15}N$, respectively.

Inspired from previous works dealing with the complexation of CN by NDA + taurine to form a 1-cyano *[f]* benzoisoindole derivative analyzed by HPLC/fluorometry, this method appears simple, rapid and reliable. LOD and LOQ for blood CN are 5 and 15 ng/ml, respectively. Calibration was found linear between 15 and 6000 ng/ml. The preliminary microdiffusion step and the MS detection make it highly specific and suitable for the analysis of badly degraded samples, *e.g.* putrefied blood. The use of $^{13}C^{15}N$ as internal standard allows the quantitation of CN with elegance and accuracy.

Keywords : cyanide, blood, HPLC/MS

The Quantitative Enzymatic Hydrolysis of Buprenorphine-3-β-D-Glucuronide in Human Urine

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Buprenorphine, a powerful analgesic as well as a substitution drug for opioids widely used in Europe and a promising new drug currently under clinical trials in the treatment of opioid dependence in the U.S., is excreted in human urine mainly as glucuronide conjugates. In the GC-MS analysis, the urine specimens must be first hydrolyzed to release buprenorphine from its glucuronide conjugates. In order to evaluate the existing hydrolysis methods and to find the optimal hydrolysis conditions, buprenorphine-3-β-D-glucuronide (B3G) was synthesized. Urine fortified with synthetic B3G was hydrolyzed using acid, base and β-glucuronidases from different source species, including *Helix pomatia*, *Escherichia coli*, and *Patella vulgata*. Glusulase®, a preparation containing both β-glucuronidase (*H. pomatia*) and sulfatase, was also tested. While both acidic and basic hydrolysis were ineffective, quantitative hydrolysis could be achieved by using β-glucuronidases under appropriate conditions. However, we found that there was a marked difference in the reactivity of these enzymes (*E. coli* > *H. pomatia* >> *P. vulgata*). The optimal incubation conditions for enzymatic hydrolysis of B3G were 2 h at 37°C for *E. coli* and 4 h at 60°C or 16 h at 37°C for *H. pomatia*. Using 1000 Fishman units of either of these two enzymes, effective hydrolysis could be achieved even when B3G concentration was as high as 2000 ng /mL. Glusulase® was equally effective toward B3G if the fortified urine samples were incubated with 25 uL of this enzyme for 1 h at 60°C.

Key Word: Buprenorphine-3-β-D-glucuronide, hydrolysis, β-glucuronidase

Cultivation and Possession of Cannabis; The Use of the Defence of Necessity and the Ethical Jury Defence in the English Criminal Courts

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The cultivation of and possession of cannabis (marihuana) are criminal offences in the United Kingdom (Misuse of Drugs Act 1971, s.5, s. 6(2)). Defendants occasionally claim that their motive for the possession or cultivation of cannabis is that it is for personal use to relieve the symptoms of a chronic medical condition, such as multiple sclerosis. They may invite the Crown Prosecution Service, to exert prosecutorial discretion, they may plead guilty and invite the judge to mitigate sentence or they may plead not guilty, admit the facts but attempt to advance the defence of necessity. That is to say, that their action is justified on the basis that, although wrong in law, it mitigates a greater evil. The use of the "ethical Jury" defence, "I did it, but the law is immoral, so you shouldn't find me guilty", is extremely rare in the English Courts. Indeed, the Ethical Jury defence cannot succeed in the face of a properly directed jury in the UK.

There is probably no general defence of necessity in English Law. A defence of duress of circumstances can achieve a similar effect in some cases. However, the case law does not suggest that that this can be applied to the circumstances where a person possesses cannabis for personal therapeutic use. Thus the defendant either has to persuade the Crown not to prosecute or to persuade the judge to mitigate sentence.

In reviewing my experience of such cases, I conclude that both the prosecution and the defence should obtain suitably qualified expert evidence at an early stage and that there must be full disclosure of the defendant's medical records. Many such cases do merit prosecutorial discretion or mitigation of sentence, but this should not be done without careful consideration of all the facts.

Keywords: Marihuana, Possession, Defence

P-16

Evaluation of cocaine adulterants apprehended in a region of Brazil.

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Cocaine, powdered or freebase form, represents a notable percentage of apprehension accomplished in the area of Campinas, S.P., Brazil. These apprehended material were conducted to Forensic Toxicology Laboratory to be analyzed qualitatively by thin-layer-chromatography. Since the beginning of 1999, forensic toxicologists observed a significant increase in the presence of lidocaine, a chemically-related local anesthetic, in these material. Then, the aim of this study was evaluated the change in the apprehension cocaine profile during the period of january/1999 and july/2000. We concluded that cocaine illicitly sold in this region was adulterated, mainly with lidocaine, followed by procaine, benzocaine and sodium bicarbonate. Results showed an increase of 245,4% in the number of cocaine samples contaminated with lidocaine in this period. This fact demonstrated that street dealers generally dilute it with such active drugs as lidocaine or procaine to increase their total sale, but the consequences of it for users or dependents have never been discussed, sometimes because there isn't enough knowledge about this contamination.

Key Words: cocaine, adulterants, apprehension

P-17

Evaluation of illicit drugs apprehension related to use and traffic in a region of Brazil.

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In 2000, illicit drugs apprehension was evaluated in the region of Campinas, S.P., Brazil, in order to verify and compare the different types and quantities involved. Marijuana is the most apprehended drug, followed by cocaine in the powdered form and cocaine in the freebase form, respectively. For all this types, the greater number of cases were related to quantity between 1,1 and 10 g, followed by cases with less than 1g. Rarely, cases of more than 1 kg of the drug were apprehended by police. By the fact that most of the cases analyzed occurred in small quantities, there is a lot of conditions and factors that must be discussed, including if the person apprehended with this drug is a user, a dependent or drug dealer, since in Brazil's Law (n° 6368/76) we have no distinctions about this categories. According to results, our proposal is to reevaluate the Brazilian Law, aiming to adjust it to our reality, mainly considering the profile of arrested person.

Key words: apprehension; drugs; Brazilian Law

P-18

The prevalence of substance abuse among university students.

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Drug abuse is a reality in any social sphere. However, official informations are mainly linked to repression, thus stigmatizing the user and reinforcing prejudice. So, this work focuses on drug abuse prevention, in order to change the present scene. To an extremely important item has been given priority, especially in prevention, which is the discussion of how extensive drug abuse is and which drugs are more frequently used by students. In the population analyzed in this study, from a total of 1686 surveys delivered to be filled out by the students, 1545 were answered, which is 91.6% of the surveys delivered, being 83.4% from females and 14.6% from males. 87.7% are financially supported by their families and 71.3% believe they have good performances at the university. From this population, **33.1%** *"have already experimented, used or are currently using"* some sort of illicit drug and 48.0% live at their parents' house. After determining how often these substances were or are currently being used, it was noted that 39.4 % of the students have used some sort of illicit drug one to six months prior to answering the survey. Among the mentioned drugs, solvent sniffing ranks first (28.4%), followed by marijuana (15,8%); however, the mixture of these two drugs, when the total use is analyzed, is responsible for the majority of cases (33.9%). The simultaneous use of perfume-squirter ("lança-perfume"), marijuana and LSD, for example, represents 1,8% of the reports.

Key words: drug abuse, epidemiology, university

P-19

Children Lead Poisoning in the Northeast of China

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Introduction: Lead poisoning affects virtually every system in the body including the central nervous system, kidney, and hematopoietic system. Very acute lead exposure in children can cause coma, convulsions, and even death. Children are more vulnerable to lead exposure than adults because of typically engaging in hand-to-mouth activities and frequently contact with lead dust in the environment. Lead poisoning has been referred to as the most important environmental hazard for children globally.

Objective: Although lead poisoning in children has been recognized as a major public health problem in China, routine screening of blood lead levels and sources of lead intoxication in children have not been established. We did epidemiological studies to determine the factors which contribute to chronic lead poisoning in children in northeast China, and to correlate these factors with blood lead levels.

Methods: We studied 77 kindergarten children, which included both girls and boys at ages 3 to 7. We also did a survey of the environmental conditions, food intake, outdoor activity, and smoking exposure of these children. Lead levels of venous blood in these children were measured by the atomic absorption method.

Results:

Blood lead levels in kindergarten Children in northeast region of China

Blood lead level μg/dL	0	0-10	10-20	20-45	>45
Number of children	5	41	13	15	3

We found that increased blood lead levels are associated with the following factors: 1). Longer outdoor activities which increased exposure to heavy air pollution from industry and motorvehicles; 2). Using coal as a major heating source; 3). Living in apartment buildings with lead-based paint; 4). Secondary cigarette smoking; 5). Family socioeconomic status, and 6). Ingestion of specially preserved eggs.

Conclusions: In our study, 36% of these children had blood lead levels from 10 to 45 μg/dL, which is higher than the allowable threshold level established by the CDC. Fifty three per cent of the children had a low but measured blood lead level (<10 μg/dL). Significant increased blood lead level was observed in 4% of the children (>45μg/ml). Only 6% of the children had an unmeasurable blood lead level. Chronic exposure and intake of lead in children remain a serious problem, especially in developing countries such as China. Although the current study was done in a region of northeast China, it represents potential lead poisoning in children nationally. In order to prevent lead poisoning in children, regulations to reduce environmental lead level should be introduced.

Key Words: Blood Lead, Children, China

P-20

Determination of Methadone, EDDP and Methadol in Breast Milk by LC-API-ES.

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Methadone is an approved pharmacotherapy for the treatment of heroin addiction. The recommendation to breast feed infants when the mother is maintained on methadone remains controversial. Methadone passively diffuses into both the aqueous and lipid fractions of breast milk. Distribution is dependent on milk composition, protein binding, acid-base characteristics and lipid solubility. Due to the presence of large amounts of lipoproteins and the lipophilicity of methadone and metabolites, the extraction of these compounds from breast milk is an analytical challenge. We have developed an assay for the detection of methadone, EDDP and methadol in breast milk using solid phase extraction (SPE) followed by LC-MS with API-ES. One mL of breast milk and 0.1 mL of methanol were mixed and centrifuged at 3500 rpm for 5 minutes and the fatty layer discarded. Sodium acetate buffer (pH 4.0, 0.2 M) and deuterated D9 methadone and D3 EDDP internal standards were added. Analytes were eluted with methylene chloride:2-propanol:ammonium hydroxide (80:20:2 v:v:v) from cation exchange/hydrophobic columns, Clean Screen DAU. Eluates were dried under a stream of nitrogen without heating, and reconstituted in 100 µL of mobile phase. Analytes were separated using a C-12 reversed phase Synergi 4 µm Max RP 80A 75 x 2 mm column. Mobile phases consisted of 55% of 2 mM ammonium acetate with 1% formic acid and 45% methanol at a flow rate of 0.25 mL/min. Extraction recoveries were 74%, 85% and 75% for methadone, EDDP and methadol respectively. Correlation coefficients for the calibration curves were $\geq$ 0.99 with a linear range of 0.5 to 500 ng/g for all analytes. Subsequent to method development and validation, we analyzed a breast milk sample from a woman maintained on methadone for her heroin addiction throughout gestation and the post-partum period. We identified in her breast milk sample methadone, EDDP and methadol. This method is being developed to analyze methadone and metabolites in breast milk obtained from methadone maintained women and compare it to maternal plasma and fetal urine concentrations. It appears this method will have application in the measurement of methadone and metabolites in breast milk.

Keywords: Methadone EDDP, Methadol breast milk LC/MS.

P-21

"A Review of Oxycodone-Related Deaths in the State of Georgia"

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Our laboratory has witnessed a dramatic increase in the number of oxycodone related deaths during the past 15 months. It appears that at least some of these of deaths may be attributable to the increased illegal use of Oxycontin®, a time-released form of oxycodone. We wish to report a number of oxycodone-related deaths that have occurred in the state of Georgia from 1999-2001. With an analgesic potency similar to morphine, oxycodone had a high abuse potential, with its products becoming common targets of drug abusers. Although drug manufacturers have incorporated oxycodone into a time- release coating to aid in the administration of oxycodone under the trade name Oxycontin®, drug abusers have been known to dissolve Oxycontin® tablets for injection in order to maximize its euphoric effects. Adverse effects associated with oxycodone in this form are similar to those of other opiates, with the most serious being respiratory depression. Destroying the outer coating can lead to rapid release and absorption of the medication and possibly result in a dangerous overdose of oxycodone.

Several recent reports demonstrate an increase in the prevalence of oxycodone abuse and oxycodone-related deaths. Recent data from the Drug Abuse Warning Network (DAWN) demonstrates that the number of oxycodone mentions in emergency department visits has been increasing over the last four years. The increased medical use and subsequent street availability of oxycodone can most likely explain this possible steady increase. Unfortunately, the DAWN data for oxycodone does not specifically mention Oxycontin® or similar product types, only those oxycodone products that contain mild analgesics (i.e. Percocet 5®, Percodan®, and Tylox®).

From 1999 to the year 2000, our laboratory saw an increase from 5 to 62 oxycodone-related cases. During the first three months of 2001, there were 23 postmortem cases that were found to contain oxycodone. It is clear that there has been an increase in the number of oxycodone-related death cases seen in our laboratory. Here, we will present several oxycodone-related cases seen in the state of Georgia over an eight-month period. Blood samples were analyzed for every case, as well as gastric contents when submitted. We also describe the procedure used for analysis and summarize the number of oxycodone-related cases seen over the past two years.

While it could not be concluded that Oxycontin® was in fact the source of the oxycodone in every case, it was listed in many of the case histories as a prescribed medication, and the majority of pills recovered from gastric contents were determined to be Oxycontin®. Judging from our data, it also could not be concluded at what levels oxycodone could prove fatal. However, our data does appear to demonstrate that oxycodone concentrations of greater than 100 mcg/L in conjunction with elevated levels of antidepressants and/or certain other prescription drugs may cause death.

Keywords: oxycodone, postmortem, Georgia

P-22

The Role of Weak Anion-Exchangers in the Extraction of Acidic Compounds

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The separation and quantitative analysis of aromatic carboxylic acids is necessary due to their importance as non-steroidal anti-inflammatory drugs and metabolites of numerous drugs and toxic substances. Solid phase extraction plays an important role in this type of toxicological analysis, because in most cases the actual determination of the acids cannot be carried out before isolating the compounds of interest from their complex environment.

Previous work has addressed factors that effect the extraction of acidic compounds using a strong anion-exchange sorbent (SAX). Results showed that the choice of counter-ion present on the anion-exchanger largely impacts the retention of acidic analytes. A generic approach to extracting acidic compounds was also developed using a mixed-mode sorbent containing a hydrophobic octyl functionality blended with SAX.

Little work has focused on the role that weak anion-exchangers have in the extraction of acidic compounds. Aminopropyl (NH_2) and propylethylenediamine (PSA) are weak anion-exchange solid phase extraction sorbents that contain one or two nitrogen functionalities, respectively. Each of these phases is different from SAX in that the degree of ionization of the nitrogen(s) is dependent on the pH of the surface environment. Results on the effect of pH on the extraction of Sulindac are shown below in Table 1.

Table 1
Results for Sulindac: pH vs. % Recovery

	% Recovery (RSD)	
PH	**PSA**	**NH_2**
2	91 (+/-3)	90 (+\-4)
5	83 (+\-2)	82 (+\-4)
7	0	23 (+\-7)
9	0	0

The data indicates that the isolation of acidic compounds using a weak anion-exchanger is dependent on the pH of the surface and analyte environments. The results also provide useful information on the mechanism of retention (i.e. hydrophobic vs. ionic or both). The pH dependence data will be presented in more detail along with the effect that the counter-ion has on the retention of acidic compounds. Mixed-mode extraction using NH_2 and PSA each blended with the hydrophobic octyl phase will also be addressed.

Key Words: Solid Phase Extraction, Ion-Exchange, Acidic Compounds

P-23

Development of a Homogeneous Enzyme Immunoassay for the Detection of Ecstasy in Urine Samples

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Ecstasy is 3,4 Methylenedioxymethamphetamine (MDMA) and belongs to a family of ring substituted methylenedioxy analogues of amphetamine. Other drugs in this family include 3, 4 – Methylenedioxyamphetamine (MDA), 3, 4 – Methylenedioxyethylamphetamine (MDEA), N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), and (3,4-Methylenedioxyphenyl) -2-butanamine(BDB). These drugs are central nervous system (CNS) stimulants that produce euphoria at low dose and hallucination at high dose. They are popular in the raves and nightclubs for their psychotropic effects and are listed by the Drug Enforcement Administration as Schedule I. The objective of this study was to develop an enzyme immunoassay for automated chemistry analyzers to screen for ecstasy and ecstasy drugs in human urine.

Microgenics ecstasy enzyme immunoassay uses a highly specific antibody that recognizes ecstasy compounds. The assay is based on competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from the urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the antibody binds the enzyme labeled drug causing a decrease in enzyme activity. Active enzyme converts NAD to NADH resulting in an absorbance change that can be measured spectrophotometrically at 340nm. The phenomenon creates a direct relationship between drug concentration in urine and enzyme activity.

The assay uses MDMA 500ng/mL as the cutoff calibrator and ±25% from cutoff as controls. The dynamic range of the assay is 0 to 1000 ng/mL. Reagents and calibrators are liquid ready-to-use. The performance of the assay was evaluated on the Hitachi 717 analyzer. Precision for intra-run and inter-run ranged from 0.9% to 1.2%. The limit of detection is 12 ng/mL and no significant interference was observed from endogenous substances. The assay is sensitive to ecstasy drugs with cross-reactivity at 67% for MDA and 116% for MDEA. The assay has minimal cross-reactivity to amphetamine compounds: Amphetamine (0.5%), Methamphetamine (0.083%), Ephedrine (0.1%) and Pseudoephedrine (0.05%). In method comparison study using GC/MS as the reference method, one hundred clinical samples were tested and the result showed 100% sensitivity and 98% specificity. Our study demonstrated that Microgenics Ecstasy Enzyme Immunoassay is a very convenient, sensitive, and specific method to screen for ecstasy drugs. The assay can be applied to many other high throughput automated chemistry analyzers.

Key words: Ecstasy, Enzyme Immunoassay, Abusive Drugs.

P-24

Volatiles by Headspace Utilizing Restek Rtx®-BAC1 and Rtx®-BAC2 Mega-bore Columns

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Instrument solutions are used to calibrate and verify the operation of breath alcohol instruments used in the state of Ohio for DUI enforcement. A method was developed to analyze ethanol in instrument check solutions for the Bureau of Alcohol and Drug Testing. Furthermore, this method was developed to utilize small sample volumes similar to those currently used in most forensic laboratories performing headspace analysis. This method investigates the performance of Resteks two BAC columns to qualitatively identify, confirm, and quantitate ethanol, methanol, acetone, and isopropanol simultaneously.

Samples were prepared by pipetting 0.1 mL of the test material and 1.0 mL of 0.02 g% n-propanol internal standard solution into a 20mL headspace vial. The vials were then equilibrated at 55°C for 18.5 min and injected into the gas chromatograph maintained at 40°C. The two columns were installed using an inlet adapter fitting kit. This modification accommodated the installation of both mega-bore columns into the injection port of the Hewlett-Packard 6890 gas chromatograph. Helium was used as the carrier gas to deliver the volatiles to two flame ionization detectors.

All volatiles achieved baseline separation with acetone and isopropanol reversing their elution order. Upper limit of linearity and limit of detection/limit of quantitation was evaluated between 0.005 g% and 0.600g% with a total of eight points used to create the curve. Linearity was demonstrated with correlation greater than 0.9995 for methanol, ethanol, acetone, and isopropanol. Precision was accessed with mixed aqueous volatile solutions tested at four levels and over five days. Review of the ethanol results for the 0.100g% solution indicate a CV% of 0.3 and 0.5 (n=10) for within day precision and 0.4 and 0.6 (n=20) for day to day precision. Serum was investigated using three levels of Bio-Rad ethanol controls over three days with the greatest variation having a CV% of 1.7 (n=15).

The combined use of these two columns offer the advantage of identification, confirmation, and dual quantitation of common volatiles with a high degree of specificity, accuracy, and precision.

Keywords: Volatiles, Headspace, Gas Chromatography, Capillary

P-25

Performance of the Roche Serum Barbiturates and Serum Benzodiazepines Assays on the COBAS Integra® 400 Analyzer

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The COBAS Integra® 400 is one of the newest members of the COBAS Integra® series of analyzers. It shares many features of the Integra® 700/800 including the consolidated workstation capabilities and a common cassette menu. In this study, we evaluated the performance of Serum Barbiturates and Serum Benzodiazepines assays on the COBAS Integra® 400. The tests are based on the Integra® Fluorescence Polarization technology. These assays were developed to yield a broad barbiturate and benzodiazepine class compound reactivity with extended dynamic range and high sensitivity. Both assays provide the flexibility of using serum, plasma or urine as samples, employing the same calibrator for all specimen types.

The within-run and total percent CVs for the Serum Barbiturates assay were less than 5 and 8 % respectively over the range of 116 - 2110 ng/mL. The within-run and total percent CVs for the Serum Benzodiazepines assay were less than 4 and 6 % respectively over the range of 26 - 145 ng/mL using NCCLS EP5-T2 guidelines. The sensitivity of the Serum Barbiturates assay was 14 ng/mL with an extended test range up to 4000 ng/mL. The sensitivity for the Serum Benzodiazepines assay was 3 ng/mL with a test range up to 200 ng/mL. This range was specifically designed for the detection of benzodiazepines at low concentration in clinical samples. Less than 10 % dose offset was detected in both methods with serum samples containing total bilirubin (≤ 23 mg/dL), triglyceride (≤ 1000 mg/dL), total protein (≤ 12.5 g/dL) and hemoglobin (≤ 9 g/L). One hundred (100) percent sensitivity and specificity was observed in method comparison trials with negative and positive serum and urine samples with the new COBAS Integra® 400 Serum Barbiturates (N = 178) and Serum Benzodiazepines (N = 188) assays vs. the COBAS Integra® 700 system. Agreements in comparisons with GC/MS confirmed positive serum and urine samples were 100 % for the COBAS Integra® 400 Serum Benzodiazepines assay and 100 % and 98 % for the Serum Barbiturates assay.

In Summary, the Serum Barbiturates and Serum Benzodiazepines assays provide reliable and acceptable performance on the COBAS Integra® 400 analyzer system with multiple specimen types.

Key Words: Barbiturates; Benzodiazepines; Fluorescence Polarization.

P-26

New Multi-analyte Rapid Screening Tests for Drugs of Abuse: OnTrak TesTstik 2 and OnTrak TesTstik 3

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The most widely abused drugs in the U.S. include marijuana, cocaine and opiates. In a recent report by Quest Diagnostics, 4.7% of the workplace drug tests showed positive results. The combined positive rate for marijuana, cocaine and opiates accounted for 84% of all positive samples (65% marijuana, 14% cocaine, and 5% opiates).

OnTrak TesTstik 2 (Cocaine/THC) and OnTrak TesTstik 3 (Cocaine/Morphine/THC) test kits are new members of Roche's On-Site DAT product line to screen for the most widely abused drugs. These in-vitro diagnostic tests are for qualitative detection of two or three drugs in urine samples at the following cutoff concentrations: benzoylecgonine and morphine at 300 ng/mL, and THC-carboxylic acid at 50 ng/mL. Both products are based on the principles of microparticle capture inhibition. The assay relies on the competition between drugs, which may be present in the urine sample, and drug conjugates immobilized on the membrane for binding to antibody-coated blue microparticles. In the absence of drugs in the urine, the antibody-coated microparticles bind to the respective drug conjugates and blue bands are formed in the result window. When sufficient drug is present, the microparticles are inhibited from binding the appropriate drug conjugate and no blue band is formed in the result window between the brackets for that drug. An additional "TEST VALID" band is formed at the end of strip to indicate that the test has completed, the reagents are viable, and the results are ready to be interpreted. The "TEST VALID" blue band forms when anti-BSA, which is immobilized on the reagent strip, binds to BSA on the microparticles.

Clinical accuracy was evaluated using a pool of clinical urine specimens that were prescreened by automated immunoassays and confirmed by GC/MS. Clinical specimens containing drugs within 25% of the immunoassay cutoff levels were also tested to challenge the product at or near the cutoff. Differences in clinical correlation between immunoassay cutoff and GC/MS cutoff will be discussed. The assay precision was determined by testing 100 replicates of six concentrations of urine standard over a five-day period. Cross-reactivity of compounds structurally similar to benzoylecgonine, morphine or THC-carboxylic acid will also be discussed.

KEYWORDS: Drugs of abuse, Screening tests, Multi-analyte.

P-27

DRUG FINDINGS IN DRIVING UNDER THE INFLUENCE OF DRUGS CASES IN VIRGINIA

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The Code of Virginia mandates that the Virginia Division of Forensic Science (DFS) operates a centralized statewide system for driving under the influence of drugs (DUID), which includes ethanol (alcohol). As such, law enforcement personnel suspecting drug induced impairment transport the suspect to a facility capable of collecting a blood specimen (typically a hospital). The blood specimen is collected under chain of custody and forwarded via U. S. mail to the DFS Central Laboratory located in Richmond. The laboratory processes the blood specimen by performing immunoassay screening for drugs of abuse. In the event of negative findings in the screen, the specimen undergoes more extensive gas chromatographic testing for prescription medications. Any positive finding requires confirmation by gas chromatography mass spectrometry (GCMS) and quantitation. Findings are recorded on the certificate of analysis which are then forwarded to the court in the jurisdiction where the traffic offense occurred.

For the year 2000, we present data on all DUID blood specimens submitted in Virginia. The ten most prevalent drugs determined from the cases submitted (n = 923) are included in the table below. A more extensive breakdown for all of the drugs found will be reported.

DRUG	N	AVERAGE (mg/L)	RANGE (mg/L)
Alcohol	353	0.08%	0.01 – 0.30
Alprazolam	108	0.09	0.02 – 1.00
Butalbital	58	10	1 – 27
Carisoprodol	17	5.5	2 – 17
Cocaine	64	0.05	0.01 – 0.3
Diazepam	84	0.37	0.1 – 2.2
Hydrocodone	20	0.06	0.01 – 0.1
Meprobamate	42	14	2 – 42
Morphine	19	0.05	0.01 – 0.15
Tetrahydrocannabinol	334	0.004	0.001 – 0.020

Key Words: DUID, impaired, drugs

P-28

Analysis of Herbal Preparations for the Presence of Kavalactones

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Kava Kava root is an herbal product that is reported to have anti-anxiety and sedative properties. It has been used as an herbal supplement or medication to treat nervous anxiety, insomnia and restlessness. Kava is available as the dried root material, in tea bags for brewing beverages, and as root extracts for direct ingestion.

Several cases have been reported in news reports of individuals that have been stopped by law enforcement personnel for investigation of driving under the influence. When a roadside sobriety test was administered, significant impairment was noted. However, after analyzing the appropriate specimens for the presence of ethanol, no ethanol was detected. After further questioning, subjects admitted to having ingested tea made from Kava Kava root.

The active ingredients in Kava Kava root are listed as kavalactones. Kavalactones can be isolated from plant material using a simple extraction procedure followed by analysis by GC/MS. In our experiments, seven different compounds were able to be identified.

Multiple sources of Kava Kava plant material from multiple vendors were analyzed to determine the relative concentration of kavalactones. Significant variations in the amount of kavalactones were seen in different preparations. Since reference standards for these compounds were not available, a semi-quantitative method was developed to estimate the concentration of each compound detected. Example chromatograms from different plant sources will be shown as well as a table of the estimated concentration of kavalactones in each material analyzed.

Key Words: Herbal Preparations, Kava Kava

P-29

An Evaluation of the OnTrak Testcup®-*er* On-Site Drug Testing Device to Qualitatively Determine the Presence of Drugs of Abuse in Urine Specimens Obtained from Emergency Departments

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We evaluated the performance of the Roche OnTrak Testcup®-*er*, an on-site urine drug test device manufactured to screen for drugs most commonly encountered in hospital emergency rooms. This on-site drug test device utilizes a competitive binding immunoassay to simultaneously determine the presence of the following drugs in urine at and above their respective cut-off concentrations: amphetamines (AMP) – 1000 ng/ml, benzoylecgonine (BE) – 300 ng/ml, morphine (MOR) – 300 ng/ml, benzodiazepines (BZB) – 200 ng/ml and barbiturates (BRB) – 200 ng/ml. One hundred forty-nine urine samples received from the emergency departments were analyzed by six different medical technologist who simultaneously tested the specimens by the EMIT® II monoclonal immunoassay and the Testcup®-*er*. Specimens yielding discordant results were analyzed by GC/MS. There was a 98% (146/149) agreement of results between the methods for the presence or absence of drugs. The Testcup®-*er* yielded 110 positive drug findings in 79 specimens; while EMIT® II yielded 102 positive drug findings in 76 specimens. There was a 97% (76/79) agreement between the methods of positive findings of at least one drug. Of the three discordant specimens that were negative for drugs by EMIT® II, one was found to contain BE, one BNZ and one BRB by Testcup®-*er*. No specimen was positive for AMP by EMIT® II, however, the Testcup®-*er* produced one false positive AMP result. Agreement on drugs detected was very good between Testcup®-*er* and EMIT® II : BE, 98% (42/43); and MOR; 97% (29/30). EMIT® II detected BRB in 8 and BNZ in 21 specimens; while Testcup®-*er* was positive for 11 BRB and 25 BNZ specimens. The discordant results were due to variations in selectivity of the assays for phenobarbital and BNZ metabolites. Testcup®-*er* demonstrated acceptable linearity by testing drug-added samples 20% below the cut-off, at the cut-off, and 20% above the cut-off.

Key Words: On-site drug testing, immunoassay, urine drug testing

P-30

Temazepam Analysis in Mother and Child

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An alleged homicide case was recently submitted to our laboratory for assistance in the toxicological analysis of the biological specimens. The case concerned a woman who was believed to have died of cyanide intoxication. A forged prescription for temazepam was also found in her residence.

Cyanide was identified in the victim's heart blood at a level of 49 μg/mL, and in her gastric contents at a level of 2.4 mg/g by gas chromatography (GC/NPD) and gas chromatography/mass spectrometry (GC/MS). Temazepam was also identified in the victim by liquid chromatography/mass spectrometry (LC/MS) at the listed concentrations: gastric contents (270 μg/mL), heart blood (1.9 μg/mL), femoral blood (1.8 μg/mL), nail clippings (2.7 ng/mg), and hair (1.4 ng/mg). Traces of oxazepam were also identified in the gastric contents and heart blood.

At the time of the incident, the victim was nursing her infant son. A soiled diaper was collected from the boy on the day of his mother's death. The diaper was hydrated with distilled water, made alkaline with ammonium hydroxide, and extracted with n-butyl chloride in a separatory funnel. The n-butyl chloride was back-extracted into dilute acid, made alkaline again, and extracted into chloroform. The extract was concentrated and analyzed by thin layer chromatography, GC/NPD and GC/MS. Control diapers were similarly analyzed. No temazepam was detected in the child's diaper, although chlorpheniramine and dextromethorphan were determined to be present. The baby's hair was negative for the presence of temazepam by LC/MS.

Key Words: Temazepam, Diaper, Hair

Trileptal®: A Postmortem Case Report

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Trileptal® (oxcarbazepine), is an anticonvulsant or antiepileptic (AED) drug, approved by the United States Food and Drug Administration in January 2000 for use as an adjunctive and monotherapy for the treatment of partial seizures. Trileptal® is chemically similar to Tegretol® (carbamazepine), but clinically different with fewer side effects. Variances include 1) fewer drug-drug interactions due to its minimal interaction with the CYP450 system, 2) no self-induction of metabolism, 3) monitoring of liver enzymes and blood parameters is not required, and 4) it is quickly and easily titrated. Oxcarbazepine can cause low levels of sodium in the blood. Signs of low levels of blood sodium include nausea, extreme drowsiness and discomfort, headache, confusion, or dullness, which can be dangerous.

A 29-year-old white, male, weighing 196 pounds, with a history of seizure disorder and no drug or alcohol abuse was found dead at home. No anatomic cause of death was found at autopsy.

Initial toxicological analysis of the aortic blood included volatiles, acidic-neutral drugs, cocaine, and opioids. An unknown peak was noted in the analysis of the acid-neutral drugs. All other tests performed yielded results of "none detected."

With a patient history of Trileptal® therapy, a presumptive match of the unknown peak was determined by gas chromatography/mass spectrometry retention time and mass fragmentation products. Consequently, Novartis Pharmaceutical Corporation (East Hanover, NJ) was contacted for oxcarbazepine and metabolite standards.

Oxcarbazepine and monohydroxy 10-hydroxycarbazepine (MHD) were confirmed and quantified by gas chromatography/mass spectrometry (GC/MS), and gas chromatography coupled with flame ionization detection (GC/FID) following a solid-support, liquid-liquid extraction utilizing Varian Chem Elut™ extraction columns, and derivatization with MTBSTFA with 1% TBDMCS. A secondary, pharmacologically inactive metabolite, 10,11-dihydro-10-hydroxycarbamazepine (DHD), was not analyzed because of gas chromatographic degradation. GC/MS quantification ions were **323**, 266, 423 for oxcarbazepine and **211**, 193, and 311 for MHD.

MHD, an active metabolite of oxcarbazepine, is also responsible for the therapeutic effect in man. The exact mechanism by which these analytes exert their anti-seizure activity is unknown. However, it is believed that they produce a blockage of voltage-sensitive sodium channels, resulting in stabilization of hyperexcited neural membranes, inhibition of repetitive neural firing, and diminution of propagation of synaptic impulses. Cytosolic enzymes rapidly reduce oxcarbazepine to MHD in the liver. The half-life of the parent drug is approximately 2 hours, while the half-life of MHD is closer to 9 hours.

In this one reported case, oxcarbazepine was detected in only trace amounts in all samples, with exception to the gastric contents, 49 mg; however, 10-hydroxycarbazepine was detected in aortic blood at 22 mg/L, in urine at 40 mg/L, and in liver at 30 mg/kg. As reported in the Annals of Clinical Biochemistry (1998), therapeutic levels of oxcarbazepine and 10-hydroxycarbazepine are 1 mg/L and 30 mg/L respectfully. The pathologist ruled this a natural death due to seizure disorder. To this laboratory's knowledge, this is the first case report of postmortem blood, urine and tissue levels for Trileptal® and its major metabolite.

Keywords: Oxcarbazepine, Antiepileptic, Postmortem

Toxicological Examination Of Thirteen Rave Party Subjects

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3,4-Methylenedioxymethamphetamine (MDMA) was originally synthesized in the early 1900s in Germany and has a chemical structure similar to methamphetamine and mescaline. As such, the drug has both stimulant and hallucinogenic properties. In the past, in the U.S. and Europe, MDMA has been used therapeutically, however, it is presently classified as a Schedule I controlled substance. The drug enjoys the designation among users as a dance or rave "club drug" and is frequently abused at "rave parties"(i.e. a high energy continuous musical party lasting perhaps all night or days). This manner of drug abuse at raves has been prevalent in Europe and is increasing in frequency in the U.S.. The Drug Enforcement Agency (DEA) reports that tablets confiscated as MDMA can contain varying amounts of the drug; generally approximately 70-100 mg. The DEA also reports that MDMA is found in combinations with amphetamine (A), methamphetamine (MA), caffeine, MDMA analogs and etc. As a stimulant, MDMA increases blood pressure, heart rate, body temperature, muscle tension, teeth clenching and sweating. As a hallucinogen, it produces a heightened sense of awareness, emotional closeness, an enhanced sense of pleasure, increased self-confidence and increased energy. Complications such as cardiac involvement, confusion, drug craving, panic attacks, depression, hallucinations and amnesia, among others, have been described.

Law enforcement officers raided a rave party in the eastern part of Virginia and thirteen subjects (n=13) were arrested, from approximately 500 who were present. The arrested subjects were found in the "chill room" wrapped together with one another "recuperating" before going back to dance. Emergency medical personnel on the scene treated those arrested, and then transported them to the hospital where each was treated. Symptoms documented on the police report includes sweating, tachycardia, jaw clenching and increased blood pressure. All subjects were calm and docile.

Our laboratory had the opportunity to perform drugs of abuse screening on the blood and urine of the thirteen subjects arrested. The average MDMA blood concentration was found to be 0.19 mg/L (range 0.09 – 0.61). Eight (8) of the subjects were found to have additional drugs in the blood. Quantitative concentrations of tetrahdrocannabinol (THC), THC carboxylic acid, benzoylecgonine, amphetamine and methamphetamine are reported. Urinary concentrations (n = 13) were found to range from 5.4 – 29 mg/L for MDMA and from 0.2 – 2.2 mg/L methylenedioxyamphetamine (MDA).

Key Words: MDMA, methylenedioxymethamphetamine, rave

Method @ Biochemical Diagnostics website

GHB - A Unique Approach in Detection and Quantitation by GCMS-EI/CI

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The objective of this presentation is to provide information for the analysis of Gamma-hydroxybutyrate (GHB) in blood or urine samples.

As rave and club scenes manage to stay alive, various types of drugs come to light. Originally sold as a health supplement, GHB has found its way into recreational use and drug facilitated sexual assaults. This drug which has gained such popularity in the underground community, has provided criminalistic and research laboratories a difficult task in its detection. GHB has eluded detection because of its structure and the ability to shift back into its equilibrium form, GBL (Gamma-butyrolactone).

Early methods for detecting GHB included analyzing samples after conversion to GBL, even the ability to detect GHB directly using solid phase extraction (SPE) of urine samples only. Later methods detected GHB in urine and blood samples which used GCMS-EI (Electron Impact). Today, studies have moved to GHB detection using SPE of urine and blood with GCMS-CI (Chemical Ionization).

DUI levels ≈ 200 µg/mL as GHB

The authors present a unique method for the detection of GHB in blood and urine samples. This method used SPE (of GHB directly), a derivatizing agent, and GC/MS. The sample was analyzed in (EI) mode as a screen for the presence of GHB. A positive sample was re-extracted with appropriate dilutions and was quantitated under (CI) mode.

The Varian Saturn 2000 GC/MS has the capability of performing CI analysis without the need for hardware modifications or gas tank changes. The CI gas consists of vapor of the solvent and can easily be changed to accommodate various reagents. Thus, analysis can be performed conveniently in either EI or CI mode without any loss of time. Full scan spectra was obtained from both EI and CI modes. The automated reports, including extracted ion chromatograms, were performed after each run. The quantitation was established based on linear calibration curves from 10 µg/mL to 1000 µg/mL. The limit of quantitation was 1.0 µg/mL. Recovery of GHB was 45.580% ± 5.000. r^2 was at 0.9998.

reagent gas ACN also 1,4-butanediol

The detection of GHB through EI is an ideal way to screen for the compound. However, a clean spectra is difficult to obtain (due to excessive fragmentation). Some attempts to use CI (with methane as a reagent gas) have been successful in obtaining a spectra, although the formation of adducts (e.g. M+29) were present. The CI method in this paper used acetonitrile as the reagent gas and gave rise to the traditional M+1 peak which is characteristic of a soft ionization method. This suggests that CI and acetonitrile work hand in hand to give a cleaner spectra.

urine has BKG levels of GHB/GBL

Actual cases that were submitted by various law enforcement agencies such as driving under the influence and sexual assaults will be discussed. This method has proven to be valuable tool in testing for GHB as time and cost are big factors in a laboratory setting.

Key Words: GHB, GBL, GCMS

detection time 6hr blood, 12 hours urine
levels of 2-10 µg/mL are grey area - could be endogenous or residue from a day before

Combined Screening and Confirmation of Drugs of Abuse in Postmortem Specimens Using LC-MS Following Solid-phase Extraction

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A simple and rapid analytical method was developed for detection and confirmation of drugs of abuse in one run. Solid-phase extraction using Bond Elut C_{18} cartridges were used to recover drugs of abuse from postmortem blood. Extracts were analyzed using high-performance liquid chromatography-mass spectrometry (LC-MS) with atmospheric pressure ionization (API) using positive mode electrospray. Chromatographic separation was achieved using a Zorbax Extend-C_{18} column with a mobile phase comprised of 0.05 M ammonia/methanol/THF (pH 9.0) (55:44.5:0.5). Mass spectrometric detection used Selected Ion Monitoring for protonated molecular ions and typical fragment ions for selected amphetamines, opiates, benzodiazepines, and/or benzoylecgonine allowed detection and confirmation of all drugs involved. Amphetamine, morphine, flunitrazepam and benzoylecgonine were selected to further validate the method. Limits of detection for all drugs were 0.01-0.05 mg/L, and recoveries ranged from 47 to 104%. Responses were linear within the range 0.05 to 5.0 mg/L. When applied to the analysis of blood and urine samples previously analyzed by conventional methodology, the method confirmed the results of the original analyses. To date, the method has been applied to the analysis of 40 drugs and metabolites.

Key Words: Drugs of Abuse, LC-MS, Solid-phase Extraction

WATLPOA

200 mg Bondelute - less clogging of column w/ post-mortem blood

Amphetamine Adducts of Melanin Intermediates Using Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry

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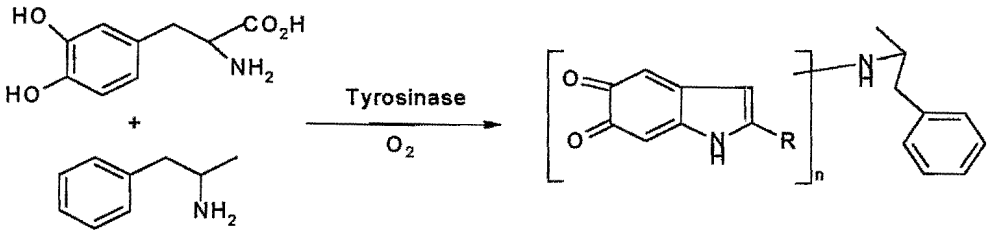
The use of hair as a matrix for the determination of a history of drug abuse is becoming increasingly widespread. Melanin has been shown to play a key role in the incorporation of drugs into hair. The mechanism of this incorporation and the nature of the interaction have remained poorly understood. Cationic drugs such as amphetamine have been thought to be ionically bound to melanin however their inextricability has lead to the suggestion that they may be covalently bound to a great degree. Identification of covalent adducts has remained elusive due to the insoluble polymeric nature of melanin. We succeeded in identifying several such adducts by Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry analysis of the products of *in vitro* synthesis of melanin in the presence of amphetamine. Amphetamine was incubated with L-dopa and mushroom tyrosinase under a stream of oxygen. After one hour a signal at m/z 281 was observed. This was identified as an amphetamine adduct of an indole quinone (n=1, R=H). Structural assignments were supported by observing the mass shifts with deuterated dopa and amphetamine analogues. Accurate mass measurements using the reflectron mode of the MS showed that these masses were within 20ppm of their theoretical monoisotopic mass. Post Source Decay measurements also supported structural assignments. After 2 hours the major adduct mass visible in the spectrum was at m/z 470. This appeared to be derived from the mono decarboxylation of a minor adduct at 514 (n=2, R=CO2H). A totally decarboxylated adduct was also observed at 424 (n=2, R=H). These adducts were also studied using deuterated L-DOPA and amphetamine adducts.



The characterization of these adducts may prove useful in understanding the mechanisms whereby drugs of abuse are deposited and retained in the hair matrix.

Key Words: Amphetamine, Melanin, Drugs in hair

Ethyl glucuronide: a biomarker for chronic alcoholism in hair?

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Ethyl glucuronide (EtG) is a minor non volatile ethanol metabolite which has been already detected by several authors in serum, urine and hair by GC-MS operating in EI mode and by LC/MS-MS. EtG has been found to be a pertinent marker for alcohol consumption that can be detected after total elimination of ethanol from body. In the present study in order to develop a method with a better limit of detection (LOD) than those published previously we used GC-MS operating in a negative chemical ionisation (NCI) mode. Furthermore, we compared our EtG results with serum alcohol concentrations, γ-glutamyltransferase (γGT) and carbohydrate deficient transferrin (CDT).

Hair specimens of 33 persons were analysed, 24 of these specimens were taken from persons at autopsy, and the other 8 specimens were provided from occasional alcohol consumers (up to 20g alcohol/day). After washing and pulverization, extraction was done by an overnight incubation in a mixture of methanol/water (1/1). After centrifugation, the supernatant was evaporated and after derivatisation with pentafluoropropionic anhydride the residue was injected into a GC/MS operating in a NCI mode. EtG (m/z=496, 342) was determined in selected ion monitoring using d_5-EtG as internal standard.

The recovery was about 60 % and the LOD was 31 pg/mg hair. In the hair of occasional users, no EtG was detected. In hair specimens taken from persons at an autopsy, 9 specimens were found positive with EtG concentrations ranging from 62 to 5800 pg/mg hair, in 6 specimens only traces were found (concentrations < LOQ), whereas in 9 samples no EtG could be detected. Values of CDT and γGT in serum from 10 of these 24 corpses varied from 2.8 to 51 % and from19 to 186 U/L respectively.

Our results point out that the use of the NCI mode in GC-MS significantly improves LOD for EtG in hair and confirm that the determination of EtG in hair provides complementary information concerning excessive chronic alcohol consumption compared to the serum alcohol findings, CDT and γGT concentrations in serum. For alcoholics a negative EtG hair result does not mean exclusion of alcohol consumption, because for example hair bleaching has to be considered. In case where EtG is detected, important alcohol consumption has to be supposed.

Key Words: Ethyl glucuronide, hair analysis, chronic alcoholism

Preliminary Analysis of Pharmacodynamic and Physiological Data After Ingestion of Oral Δ^9-Tetrahydrocannabinol

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The increasing availability of food products containing Δ^9-tetrahydrocannabinol (THC), and synthetic THC medications has necessitated the need for a controlled study of oral THC's psychoactive and physiological effects. The NIDA Institutional Review Board approved this study and each subject gave informed consent. During the study, all subjects resided on the NIDA IRP research ward, under continuous medical surveillance. The study was designed as a randomized, double blind, double dummy, placebo-controlled within-subject protocol. The participants were dosed three times a day, with meals, for five consecutive days followed by a ten-day washout period before the next dosing session began. Seven (N=7) healthy volunteers with a history of marijuana abuse ingested commercially available hemp seed oils of differing THC concentrations: 0, 9, 92, 347 μg/g, in liquid or capsule form, for total THC dose per day of: 0, 0.39, 0.47, and 14.8 mg; 7.5 mg of Dronabinol was used as a positive control. Dronabinol, Marinol®, a synthetic THC medication was administered in a 2.5 mg capsule. Performance and physiological measures were assessed before the first dose and repeatedly over the dosing period.

A computerized logical reasoning task was performed to measure verbal information processing. Data, percent correct answers and response time, were analyzed using a repeated measures analysis of variance (ANOVA) with dose and time as factors. Preliminary results indicate no significant main effects or interactions. The analysis did, however, reveal a trend toward a trial main effect on response time ($p = .073$) for the total dosing period, and a trend toward a dose main effect on percent correct responding ($p = .074$) for the four consecutive trials on the first day of dosing.

Subjects' heart rates were monitored throughout the dosing period. Change from baseline heart rate data were analyzed and no significant main effects or interactions were indicated. Once again, trends towards a trial main effect ($p = .081$) and interaction ($p = .078$) of the two main effects for dose and time were noted.

These preliminary results indicate no significant increase in heart rate or decrease in cognitive performance on the logical reasoning task following ingestion of 0.4 to 14.8 mg THC per day. This is not unexpected due to THC's poor bioavailability (approximately 6 - 20%) via the oral dosing route.

Key Words: Marijuana, Hemp Oil, Oral THC, Dronabinol, Heart Rate, Cognitive Performance

OXYCODONE CASES OF DUID IN VIRGINIA

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Oxycodone is a drug which is classified as a semi-synthetic opiate, and is derived from codeine. Oxycodone, a Schedule II controlled substance, is an ingredient of prescription drugs such as Percodan®, Percocet®, Roxicet®, Tylox®, etc. The prescription usually appears in combination with other non-narcotic and less addictive drugs (e.g. aspirin, acetaminophen). Principal actions of oxycodone (OC) include analgesia (pain relief) and sedation. In the recent past, the drug was typically administered orally in a 2.5 to 5 mg dose every six hours for the relief of moderate to moderately severe pain. More recently in 1995, OC was introduced as the single entity drug OxyContin® and significant changes occurred in how OC was (is) formulated. Significant changes now noted are that OxyContin® is: a single entity agent (i.e. pure drug); formulated as a time release preparation (12 hour); now sold in 20, 40, 60, 80, and 160 mg doses. The changes produce numerous beneficial effects and increase the number of legitimate patients administered and helped by this medication. At the same time abuse has increased, and the increase in both legitimate and illegitimate use results in more drivers on the road with OC in their blood.

OC is considered a narcotic, and as such, has the addictive and/or abuse potential of morphine. When consumed in sufficient quantity, OC is capable of producing stupor, coma, muscle weakness, low and/or severely depressed respiratory rate, hypertension, and cardiac arrest in overdose. There are various degrees of toxicity, related to the dose (i.e. the higher the dose the more pronounced the symptoms). With less than toxic doses, some of the less severe adverse reactions include lightheadedness, drowsiness, dizziness, nausea, vomiting, and pupillary constriction. There are patient warnings related to OC noting that "OC may impair mental and/or physical abilities required for the performance of potentially hazardous tasks such as driving an automobile or operating machinery. The patient using this drug should be cautioned accordingly." In this study, we investigate 29 cases of driving under the influence of drugs (DUID) where OC was identified in the blood of drivers. Blood OC concentrations ranged from 0.01 to 0.50 mg/L. Blood concentrations were quantitated by gas chromatography (GC) using a nitrogen detector, and qualitatively by GC mass spectrometry (GCMS). Symptoms as noted by the police officer and recorded on the field sobriety test (FST) are discussed as well as statements by the driver. Additional cases will be added as data is accumulated

Key Words: oxycodone, DUID, effects

P-31

Drinking and driving: perception of intoxication and estimated blood alcohol concentration of drinkers in social settings.

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In January 1998, the Brazilian Road Traffic Act has been changed and established a legal blood alcohol limit of 0,6 g/l. However, lack of information about effects of alcohol and number of drinks they need to consume to get drunk or to reach the legal limit predominate between the users. Randomly selected individuals consented to be interviewed as they walked by preselected drinking establishments, and afterward provided a breath sample to determine BAC. Approximately 51% of the people intended to drive away themselves, while 49% were driven by others. People's decision whether or not to drive was unrelated to their BAC and to the number of drinks they reportedly had. Mostly of that who intended to drive away themselves had a BAC superior to legal limit. The average BAC was 0,71 g/l for all cases. Although, a legal restriction about drinking and driving exists, individual variability and ignorance cause disregarding to the law. Then, prevention programs are extremely necessary to decrease the numbers of alcohol-related accidents.

Key Words: drinking, driving, social settings

P-32

Liquid Chromatographic - Electrospray Ionization - Tandem Mass Spectrometric Method for Determination of LAAM, norLAAM and dinorLAAM in Human Plasma and Urine

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l-□-Acetylmethadol (LAAM) is an alternative to methadone for the maintenance treatment of opiate use. LAAM has a longer therapeutic half-life than methadone, primarily because it is metabolized to more active metabolites norLAAM and dinorLAAM. Although FDA-approved, further research is required on its pharmacokinetics, metabolism, potential drug interactions and toxicity in humans and laboratory animals. To support this research, sensitive and selective analytical methods are needed to monitor the concentrations of LAAM and its metabolites. While gas chromatography /mass spectrometry (GC/MS) methods have adequate sensitivity to detect LAAM and metabolites in samples from maintenance patients, it may not be useful for lower doses in experiments with opiate non-tolerant individuals and GC/MS analysis of LAAM and metabolites also requires chemical derivatization. This study describes a liquid chromatography - tandem mass spectrometry (LC-MS-MS) method capable of detecting LAAM and its metabolites, norLAAM and dinorLAAM. This assay has a linear range of 0.25 - 100 ng/mL for the three analytes. Inter-and intra-assay precision varied by less than 9% for all analytes at 0.25, 0.5, 5 and 70 ng/mL concentrations. The inter-and intra-assay accuracy for all analytes at lower limit of quatitation and other concentrations were within 18% and 13% of target, respectively. All analytes had acceptable stability after three freeze-thaw cycles, or at room temperature for 20 hour, or after storage of extracts either at -20°C for 6 days or on the autosampler (10°C) for 4 days. This research was supported in part by R01 DA10100 .

Key words: LAAM, Metabolites and LC/MS/MS

P-33

Does Creatinine Normalization Decrease the Variations in Urinary Levels of Endogenous GHB?

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Gamma hydroxybutyrate (GHB) and its precursors (gamma butyrolactone (GBL) and 1,4-butanediol) have become popular recreational drugs. Further, their short half-lives and strong amnesiac and sedative effects make them likely candidates in drug-facilitated sexual assault (DFSA) cases. One of the difficulties surrounding the interpretation of GHB and its precursors in these cases is the natural presence of GHB in mammalian blood and urine. Therefore, forensic toxicologists investigating DFSA cases are often called upon to differentiate between endogenous and exogenous concentrations of GHB in biological specimens. This study was designed to determine the variation in urinary levels of endogenous GHB over a one-week period and whether those variations are reduced by normalization to creatinine.

The NIDA Institutional Review Board approved the protocol, and subjects provided informed consent and resided on the closed research ward under continuous medical surveillance. Every urine void produced by eight GHB-free subjects (5 males and 3 females) over a one-week period was individually collected and quantitatively analyzed for the presence of endogenous GHB and creatinine. GHB analysis was carried out using a published headspace GC/MS analytical procedure. Creatinine was analyzed on a Beckman Synchron CX7 Delta System.

The unnormalized data ranged from 0.00-6.63 mg/mL with mean concentrations as low as 0.28 mg/mL and as high as 3.02 mg/mL. The CVs for the unnormalized data ranged from 44.0% to 79.4%. When the data was normalized to creatinine, the concentrations ranged from 0.00-6.68 mg/mg with mean concentrations as low as 0.32 mg/mg and as high as 3.48 mg/mg. The CVs for the creatinine-normalized results were between 29.7% and 81.3%.

The results of our study suggest small improvements in the CVs of 7 of the 8 subjects when the concentrations are normalized to creatinine. Further analysis of the data (paired t-test; a=0.1) suggests these improvements are statistically insignificant. However, the findings do substantiate the proposed cutoff of 10 mg/mL (or 10 mg/mg) to differentiate endogenous from exogenous GHB.

Keywords: GHB, endogenous, drug-facilitated rape

P-34

Partition Characteristics of Aliphatic Alcohols when Measured from Biological Specimens Using Headspace Gas Chromatography

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The impact of partition coefficient variation upon quantitative results of aliphatic alcohols when measured from biological samples using headspace gas chromatography (HS-GC) was investigated. Alcohols partition into tissues based upon the water content of the tissue and the carbon content of the alcohol. Conversely, during analysis, any uncorrected partition coefficient variation between calibrators and the biological specimens being analyzed can bias the quantitative results of HS-GC techniques.

The partitioning of methanol, ethanol, n-propanol and n-butanol from different biological matrices was initially investigated by comparing peak area measurements of spiked tissues and body fluids to aqueous samples of identical concentration. Partitioning of alcohols from biological samples was found to statistically differ from aqueous samples, when using *t* to estimate the distribution of peak area ranges.

Additionally, a quantitative method employing n-propanol as an IS was evaluated. Linear regression curves derived from aqueous calibrators were used to calculate the apparent amount of methanol, ethanol and n-butanol in serum, blood and brain samples. Relative to n-propanol, the less lipophillic methanol and ethanol yielded erroneously high results. Methanol concentrations were overestimated in all biological specimens, being as much as 13.6% above target when measured from brain. Ethanol levels were as much as 4.1% high in brain samples. The more lipophillic n-butanol was underestimated in all samples, being as much as -8.0% from target when measured from brain tissue.

The results are in agreement with the hypothesized effects of partition coefficient variation. Therefore, it is proposed that laboratories examine their HS-GC methods for potential error or matrix match their calibrators and controls to the samples being analyzed.

Key words: Headspace gas chromatography (HS-GC); Partition coefficient; Aliphatic alcohols.

P-35

A Retrospective Analysis of DUI Drug and Alcohol Concentrations from a Large Suburban County

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A retrospective analysis of driving under the influence cases was conducted. The data represents a tabulation of drug and alcohol concentrations found in 7384 DUID cases submitted to National Medical Services from 1996 through 2000. Almost exclusively the cases represent the surrounding suburban area of Philadelphia. Cases were analyzed for demographic information, drug and alcohol concentrations. Appropriate descriptive statistics were applied to the data and the results presented in a tabular format.

Of all the cases submitted, 87% contained age and sex information of the individual stopped for perceived impaired driving. Of those cases, 81% involved males and 19% female. The mean age for those cases containing demographic information was 34.5 years with a standard error of the mean of 0.1 year. The age range was 12 to 88 years. The median age for this group was 33 years. Analysis of the age groups revealed the 25- to 30-year-old age group had the highest incidence of DUI arrests with the 31- to 35-year-old range being the second largest group. The 19- to 24-year-old group had the lowest incidence of DUI arrests. Each DUI submission to the laboratory was subject to immunoassay, gas chromatography and gas chromatography-mass spectrometry. The drug found to be in the highest frequency in DUI positive cases was not surprisingly ethanol, followed by cannabinoids. 94% of cases were positive with either ethanol alone or in combination with other impairing agents. The mean ethanol concentration for all ethanol positive cases was 174 mg/dL (range 0-481 mg/dL). Another 6% of the cases were negative for the presence of ethanol but had either a single or multiple potentially impairing agents. Further analysis of cases without ethanol indicated that 50% of the cases contained cannabinoids followed by cocaine (20%) and benzodiazepines (14%). The remaining 16% of ethanol negative cases involved in descending frequency; opiates (9%), methadone (3%), phencyclidine (1.5%), amphetamines (1%), propoxyphene (1%) and others (0.5%). Analysis of the benzodiazepine group for all DUI positive case showed that diazepam and nordiazepam (35%) had the highest incidence followed by alprazolam (14%). In the amphetamine group, MDMA had the highest incidence (26%) followed by phentermine (21%). For the opiate group, morphine (53%) had the highest incidence. Hydrocodone and oxycodone had an incidence rate of 17% and 12% respectively. Concentrations of other substances found in the cases and the associated descriptive statistics were calculated for presentation.

Keywords: DUI, Alcohol, Statistics

P-36

Analysis of Δ-9 Tetrahydrocannabinol and Metabolites in Blood using Cerex PolyChrom-THC™ Solid Phase Extraction Columns and Tandem Mass Spectrometry

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Driving under the influence of marijuana continues to be a major traffic safety problem in California. In 2000 approximately 45% of the driving cases analyzed at this laboratory were positive for marijuana. Marijuana blood levels decrease to very low levels in a short period of time after smoking. The analysis of this drug in blood requires a high level of sensitivity and specificity.

This laboratory has previously utilized a liquid-liquid extraction procedure, which required a large commitment of both time and resources. We have developed an analytical method that uses a Cerex PolyChrom-THC™ solid phase extraction column that requires no preconditioning and a minimal amount of sample preparation. The method consists of the extraction of 1 milliliter of blood with deuterated internal standards, conversion to a silyl derivative and analysis on a gas chromatograph/tandem mass spectrometer (GC/MS/MS) in the electron ionization mode. The two major analytes of marijuana, Δ-9 Tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ-9 Tetrahydrocannabinol (COOH-THC) are detected by GC/MS/MS in the single reaction monitoring (SRM) mode. Limits of detection for THC and COOH-THC are 0.3 and 0.04 nanograms/per milliliter (ng/ml) respectively and the limits of quantitation are 1.3 and 0.09 ng/ml respectively. The method was linear at concentrations from 0.1 to 100 ng/ml with R^2 values greater then 0.99. Recovery of THC and COOH-THC by this method was at 72 and 82% respectively. Intra-assay precision was determined by the analysis of known quality control material (n=4) with coefficients of variation for THC and COOH-THC at 6 and 8% respectively.

This extraction procedure, combined with the sensitivity and specificity of tandem mass spectrometry, has increased this laboratory's ability to efficiently detect low levels of THC in the blood samples of impaired drivers.

Key Words: Cannabinoids, Tandem Mass Spectrometry, Solid phase extraction

P-37

Influx and Efflux of Amphetamine and N-acetylamphetamine in Keratinocytes, Pigmented Melanocytes, and Non-Pigmented Melanocytes.

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In relation to the potential hair incorporation color bias for certain basic drugs, a basic drug (amphetamine), its non-basic analog (N-acetylamphetamine) were analyzed for influx and efflux into and out of keratinocytes, pigmented melanocytes (PM), and non-pigmented melanocytes (NPM) as a model for incorporation and efflux of these drugs from hair cells. NPM were of the same melan-a cell line as PM, but cultured in the presence of the tyrosinase inhibitor phenylthiocarbamide. Influx methods involved 1) growing cells to near confluency in 25 cm^2 flasks 2) adding drug-containing media for an allotted period of time 3) quickly rinsing the cells three times with ice-cold phosphate buffered saline 4) lysing the cells 5) performing a protein assay on whole cell lysates to normalize data for cell content and 6) extracting and analyzing drug taken up via LC/MS/MS. Efflux methods included adding drug-free media to cells after influx and rinsing, collecting media after an allotted time period, and analyzing collected media and cells for drug content via LC/MS/MS.

Results show that PM take up large amounts of the basic drug amphetamine (levels of uptake dependent on melanin content), while keratinocytes and NPM take up only small amounts of amphetamine. None of the cells take up N-acetylamphetamine above background levels. Interestingly, while keratinocytes and NPM quickly efflux most of the influxed amphetamine, PM are slow to efflux and only efflux ~65% of influxed drug, if efflux media is not refreshed. (If efflux media is periodically refreshed, PM will eventually redistribute essentially all amphetamine back into the media.) In conclusion, these results demonstrate that pigmented cells take up greater amounts of amphetamine and efflux it more slowly than non-pigmented cells. This is consistent with previous data for in vivo incorporation of amphetamine in animal hair. Analogous drug transport may occur with other basic compounds as well. Overall, the data suggest that individuals with pigmented hair can be expected to incorporate more basic type drugs into their hair than persons with non-pigmented hair. Supported by NIH grants DA07820 and DA 09096.

Key Words: Hair testing, melanin, amphetamine

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Determination of Gamma-Hydroxybutrate (GHB) and Related Substances in Forensic Samples by LC/MS

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Gamma-Hydroxybutrate (GHB) has gained notoriety in recent years as both a club and "date rape" drug. Following the classification of GHB as a regulated substance, analogs have emerged which can be both metabolically and chemically converted to GHB. A challenge for the forensic science community is to develop a fast, simple and reliable method for the simultaneous analysis of GHB and it's most prevalent analogs, Gamma-Butyrolactone (GBL) and 1,4-Butandiol (1,4-BD). A novel method for detecting and quantifying GHB, GBL and 1,4-BD by LC/MS in forensic samples is reported.

The extraction/preparation steps used were dependant on the sample matrix. Unlike many of the previously reported methods for the analysis of GHB this method does not involve the conversion of GHB to GBL or derivatization prior to analysis. The analysis method developed involves the separation of the analytes by LC with electrospray – MS detection. Quantitation was performed using external standards and the method was linear in the range required for the analysis of both adulterated substances and biological samples. The detection limit was approximately 0.3mg/L for all three compounds.

Key Words: Gamma-Hydroxybutrate, Forensic Samples, LC/MS

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Influx and Efflux of Cocaine in Keratinocytes, Pigmented Melanocytes, and Non-Pigmented Melanocytes

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There is increasing evidence that drug basicity and charge at physiological pH strongly affects binding to the melanin in hair. A common basic drug (cocaine, 1μM) was analyzed for uptake and efflux into and out of pigmented melanocytes, non-pigmented melanocytes and keratinocytes. Pigmented melanocytes (mouse melan-a cells) were cultured in the presence (+) and absence (-) of the melanin inhibitor phenylthiocarbamide (PTC). This procedure gave rise to pigmented and non-pigmented melanocytes of the same cell line. Keratinocytes were grown in the absence of phenylthiocarbamide in its own media. In uptake (influx) experiments, all cells were: 1) cultivated in 25 cm^2 flasks to ~90% confluency; 2) dosed with media containing cocaine and allowed to incubate for a fixed time, and; 3) rinsed with 5 ml of chilled phosphate buffered saline (3x to insure that all drug containing media had been washed away). Cells were then lysed and a protein assay performed to normalize data for cell content. Cocaine influxed by the cells was analyzed via LC/MS/MS. Efflux protocols were identical to influx protocols except that blank media was added to dosed cells, incubated for an allotted time, collected, and assessed for redistribution of cocaine from the cells to the media.

Repeated influx experiments consistently showed that pigmented melanocytes (-PTC) take up and retain far more (up to 11x more) cocaine than non-pigmented melanocytes (+PTC) and keratinocytes. The higher melanin content in the pigmented melanocytes correlated with a higher concentration of cocaine remaining in the cells and lower concentrations of cocaine found in the efflux media. After blank media is introduced, most of the cocaine taken up by non-pigmented melanocytes and keratinocytes is quickly, and almost completely, released back into the media. These data are consistent with previous *in vivo* research examining incorporation of cocaine in animal hair. These data suggest that persons with darkly pigmented hair, given an equivalent dose, are more likely to incorporate basic drugs into their hair than those with a less or non-pigmented hair type. Supported by NIH grant DA09096

Screening and Confirmation of Cyanide in Biological Matrices by Headspace GC-NPD and Headspace GC-MS (EI).

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Cyanide is a potent and rapidly acting poison. It is also detected in healthy individuals at low concentrations, typically less than 0.26 mg/L in blood. An analytical procedure for the screening and confirmation of cyanide in biological matrices was developed for use in the FBI Laboratory. Specimen preparation for both procedures involves adding 1 mL of aqueous calibrator, control or unknown specimen and 100µL of internal standard solution to a headspace autosampler vial, which is then sealed. One (1) mL of 5N sulfuric acid is injected through the septum of each vial to liberate HCN. The vials are vortexed and loaded onto the headspace autosampler for analysis. The analytical parameters for each method follow:

GC-NPD Analysis		GC-MS Analysis	
Column Type:	Packed; Porapak Q5	Column Type:	DB-624
GC Temp Prog:	100°C isothermal	GC Temp Prog:	60°C for 2 min; ramp to 120°C at 50°C/min; hold for 7 min
Internal Standard:	0.04% acetonitrile	Internal Standard:	0.1% 1-butanol
LOD:	0.25 mg/L	LOD:	0.50 mg/L
Linearity:	0.25 – 10 mg/L	Linearity:	1 - 50 mg/L
Accuracy:	+0.1±0.11 at 1 mg/L (N=5) +2.1 ±0.35 at 10 mg/L (N=4)	Accuracy:	0 ±0.12 at 10 mg/L (N=13) +1.3±0.26 at 25 mg/L (N=13)
Precision:	cv< 10% 1mg/L (N=5) 10 mg/L (N=4)	Precision:	cv< 10% 10 mg/L (N=13) 25 mg/L (N=13)
		GC-MS SIM Ions:	12,26,27

Hydrogen cyanide elutes within 2 minutes in both procedures. For a more specific analytical result, the GC-MS method may be utilized in the full scan mode, with adequate sensitivity to detect 0.50 mg/L. The use of headspace GC-NPD in combination with headspace GC-MS provides a sensitive and specific analytical procedure for the screening and confirmation of cyanide in biological specimens.

Key Words: cyanide, headspace GC-NPD, headspace GC-MS

P-41

Stability of Blood Alcohol Samples Over Time

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A study was undertaken to examine what impact the lack of refrigeration might have on stored blood alcohol samples. Does room temperature storage of preserved forensic blood alcohol samples have an effect on the concentration of ethanol and other low molecular weight volatiles? The volatiles used for this study included ethanol, methanol, 2-propanol and acetone.

Ten (10) human blood samples were preserved by addition of 1% NaF. Various amounts of the volatiles were added to the blood samples and thoroughly mixed. Qualitative and quantitative analyses were performed using headspace gas chromatography (Varian 3400 with a Carbowax on Carbopack C column). The samples were analyzed when initially prepared and were then refrigerated for 104 days. Samples were removed from refrigeration, maintained at room temperature and subsequently analyzed on the 133rd, 264, 334, 448 and 560th days after preparation.

The samples containing ethanol showed a consistent, though variable tendency to decrease in concentration. Those samples to which no ethanol was added, continued to be ethanol-free throughout the length of the study. Concentrations of the other volatiles (methanol, 2-propanol and acetone) demonstrated a small but consistent loss of these compounds with time.

Forensic blood samples preserved with 1%NaF appear to maintain stable to decreasing alcohol concentrations for several months even without refrigeration. Lack of refrigeration of these samples did not result in production of ethanol or other volatiles.

Key Words: Blood Alcohol, Time, room Temperature

P-42

Evaluation of a Micro-Plate Enzyme Immunoassay (EIA) in Screening Postmortem Blood Specimens for the Presence of Fentanyl

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Fentanyl is a short acting, synthetic narcotic analgesic of high potency with strong abuse potential. The drug is available as an injectable solution or as a transdermal patch and administered as an adjunct to anesthesia and for management of chronic pain. Qualitative and quantitative detection of fentanyl in biological specimens can be a challenge due to its low dose administration. Radioimmunoassay (RIA) has been classically used for this determination. A sharp spike in the price of RIA kits and an increase in the incidence of illicit fentanyl use led our laboratory to investigate alternative screening techniques.

OraSure Technologies, Inc. (Bethlehem, PA) has developed a micro-plate enzyme immunoassay (EIA) for quick screening of biological specimens for the presence of fentanyl. The assay depends on the competition between free drug in the specimen and drug bound to enzyme for an antibody (rabbit) which is fixed to 96-well polystyrene plates. The instrumentation utilized for screening is a BioChem Personal Lab autoanalyzer (Allentown, PA). The cut-off concentration for this procedure is 1 ng/mL. For the purposes of screening whole-blood postmortem specimens, the specimens, calibrators and controls are first diluted 1:5 with forensic specimen diluent then analyzed according to the manufacturer's guidelines. Whole-blood calibrators and controls were prepared in-house and verified by gas chromatography/mass spectrometry (GC/MS). In addition, the negative and cutoff serum calibrators supplied by the manufacturer are analyzed and the percent displacement of absorbance is monitored for quality control purposes.

For the purposes of this investigation, 426 medical examiner cases were screened for fentanyl utilizing the above commercially available EIA kit supplied by the manufacturer. Specimen selection criteria included (1) death occurred during the second quarter of 2000 (2) decedent age was >15 and <75 years and (3) there was sufficient specimen volume (2 to 4 mL) to screen and confirm results.

Samples exhibiting a positive response were confirmed by GC/MS following a solid phase extraction procedure utilizing United Chemical Technologies Clean Screen® columns (Bristol, PA). Monitoring ions of fentanyl included **245**, 146, 189, and for fentanyl-d5 were **250**, 151, 194 (quantification ions in bold).

Of the specimens tested 16 (3.8%) were presumptively positive using the fentanyl kit. Of these, twelve cases (75%) were subsequently confirmed to be positive for fentanyl by GC/MS. Quantitative values for these cases ranged from 2 to 49 ng/mL. Only six of these twelve cases were expected to be positive based on known fentanyl use. The four presumptive positive cases not confirmed by GC/MS are currently being investigated for the presence of fentanyl analogues that may cause cross-reaction with this kit. Four additional cases showed reactivity between the negative control (0.5 ng/mL) and the negative calibrator (0 ng/mL) and are also being investigated for cross reactivity. Between run cv's ranged from 4-12% and all runs passed quality control criteria.

The results from this population study demonstrate that the OraSure Technologies, Inc. EIA fentanyl kit shows satisfactory performance in this postmortem forensic toxicology application. This screening technique offers laboratories cost efficiency, rapid and simple specimen preparation, and an acceptable confirmation rate of specimens positive by EIA.

Key Words: Post-mortem, EIA, Fentanyl

P-43

Tissue Distribution of Temazepam in a Fatal Overdose

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A case is reported in which a lethal level of temazepam and asphyxia by a plastic bag was determined to be the cause of death. The decedent was a 43-year-old female found in the cargo area of her sport utility vehicle parked at a nearby grocery store. Significant findings included a plastic grocery bag over her head, with the handles loosely tied around her head; acute, passive congestion of the liver, kidneys, and lungs; multiple notes detailing her intent to commit suicide were found in the vehicle; and an elevated temazepam concentration was detected in postmortem blood. The decedent had a history of depression and had attempted suicide on four previous occasions using her medications. Temazepam was identified and quantitated in the following tissues: heart blood – 7.8 mg/L, urine – 4.2 mg/L, muscle – 5.9 mg/Kg, liver – 15.5 mg/Kg, kidney – 11.3 mg/Kg, and brain – 7.5 mg/Kg. Methylphenidate was also detected in the urine (0.14 mg/L) and at trace levels in the heart blood and all submitted tissue specimens. Temazepam (Restoril®), a benzodiazepine hypnotic, is generally considered to be a very safe drug with a wide therapeutic margin. Although fatal drug intoxications involving multiple drugs, including temazepam, have been previously documented, there are only a few documented fatalities in which the determination of an elevated temazepam concentration was the only significant toxicological finding; extensive tissue distributions of the drug were not performed in these cases. In the fatality reported here the cause of death was determined to be temazepam intoxication and asphyxia by plastic bag and the manner of death was ruled to be suicide.

Key Words: Temazepam, Postmortem Distribution, Drug Intoxication

P-44

Interpretation of Drug Concentration Following Post-Mortem Tissue Degradation

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Interpretation of tissue drug concentrations often involves a comparison with values found in the literature which are based on the analysis of fresh specimens. However, post-mortem degradation, particularly that which occurs with prolonged exposure to the elements, can result in significant tissue changes that may alter the interpretation of tissue drug concentration. In a recent case analysis, a liver specimen was found to have a diphenhydramine concentration of 6.6 mg/kg tissue, a result consistent with toxic diphenhydramine ingestion. However, the history of the specimen suggested that this concentration might not lead to an appropriate interpretation of the peri-mortem diphenhyramine concentration. Specifically, the documentation accompanying the specimen stated that the decedent was exposed to the impact of a vehicular crash and subsequent fire. The remains of the decedent were then exposed to the elements for approximately 23 days before recovery.

The protein and iron concentrations of the degraded liver specimen were compared to reported literature values and to those of a fresh liver specimen using spectrophotometry and atomic absorption spectroscopy, respectively. Analysis of tissue protein concentration indicated an approximately two-fold difference between the degraded specimen (0.19 mg/mg tissue) and the fresh tissue (0.10 mg/mg tissue). However, the use of total protein is only valid if factors affecting the sample do not result in degradation of protein itself. Measurement of iron concentrations in the two specimens resulted in significant concentration differences. The iron concentration of the fresh liver (117 mg/kg tissue) was consistent with the reported concentration range of 29-240 mg/kg tissue (1). In contrast, the iron concentration of the degraded liver was 1070 mg/kg tissue or 9.1 times greater than the fresh liver iron concentration.

Based on these results, the iron concentration of the degraded specimen was used to account for post-mortem changes in mass in order to better approximate the peri-mortem diphenhydramine concentration. Using ratios of liver and blood diphenhydramine concentrations found in scientific literature suggested that the peri-mortem diphenhydramine concentration could be nearly an order of magnitude less than the concentration determined after the fire and prolonged post-mortem interval. These results indicated that iron may be superior to protein for establishing post-mortem changes.
(TAH was supported in part by the 2001 Internship Grant at the American Institute of Toxicology.)
1. R.C. Baselt. *Disposition of Toxic Drugs and Chemicals in Man*, 5th ed., 2000, p 444.

Key Words: tissue, drug concentration, post-mortem changes

P-45

An Evaluation of Oral Fluid Drug Testing for the detection of Opiate and Cocaine use in a population of Drug Users

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New legislation has been introduced in the UK that provides statutory powers to allow drug testing of offenders at various stages of the Criminal Justice process. The legislation specifically requires a point-of-contact test and makes provision for either urine or a non-intimate sample such as oral fluid specimens to be analysed for the presence of opiates and cocaine / benzoylecgonine. The use of an electronic test reader to obviate human subjectivity in deciding between positive and negative specimens was seen as desirable.

An evaluation was undertaken that included the collection and analysis of 1100 oral fluid samples and 709 urine samples from volunteers attending drug treatment centres. Point-of-contact oral fluid testing was undertaken using the Cozart RapiScan system. Oral fluid samples were subsequently reanalysed in the laboratory using a second RapiScan, microplate immunoassay and GC/MS. Urine samples were screened in the laboratory using an Olympus AU600 Analyser and Roche On-Line reagents.

The paper will provide an overview of the analytical results obtained from the different analytical procedures, a comparison of results obtained from the analysis of oral fluid and urine, the windows of detection for opiates and cocaine in the population tested and the comments gathered from collectors and donors concerning the use of oral fluid as a medium for drug testing.

Keywords: Oral fluid, Opiates, Cocaine.

Methadone Findings in Drivers and Post Mortem Cases in Washington State

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In Washington State, as perhaps elsewhere, there has been a strong interest in expanding methadone maintenance programs. In addition, physicians have increasingly prescribed methadone for chronic pain. Some of the medical examiners in Washington State have been questioned when they attribute a death to methadone toxicity because of a concern that it might adversely affect efforts to expand methadone maintenance programs. Because we perform the toxicological analysis on both a driving population and a post mortem population at the Washington State Toxicology laboratory, we decided to compare the findings for these two populations. Over a 16-month period, we had 236 cases with methadone as a finding. 176 cases were from post mortem examinations, 59 were drivers and 1 was a sexual assault victim. The post mortem cases had a mean concentration of 0.48 mg/L methadone (median 0.28, span <0.05 – 5.3 mg/L). The 95% confidence interval for the mean was 0.39 – 0.58 mg/L. The driving population had a mean concentration of 0.20 mg/L methadone (median 0.15, span <0.05 – 1.29 mg/L). The 95% confidence interval was 0.14 – 0.27 mg/L. 92% of the death investigation cases and 98% of the driving cases had other CNS active drugs present. A small subset of samples was analyzed for the major methadone metabolite, EDDP. EDDP was found in concentrations just under 10% of the parent drug and there was no significant difference in metabolite to parent drug ratios in this small subset between drivers and post-mortem cases.

Keywords: methadone, EDDP, post mortem

Detection of Opiate Use in a Methadone Maintenance Treatment Population with the CEDIA® DAU 6-Acetylmorphine and CEDIA® DAU opiate assays

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6-acetylmorphine (6AM), a specific marker for heroin use, has a short detection time (t1/2 0.6h) in urine. Ingestion of poppy seeds and licit opiate analgesics also produces positive opiate tests, making interpretation difficult. Opiate cutoff concentrations recently increased and 6AM analysis added for the federal workplace drug-testing program. Microgenics™ Corporation (Fremont, CA) has developed the CEDIA®# DAU 6AM assay for qualitative and semi-quantitative determination of 6AM in urine. Urine specimens, collected 3 times per week from 27 individuals enrolled in a methadone maintenance treatment program, were screened for opiates and 6AM and confirmed by GC/MS. Of 1377 specimens tested for opiates, 18.9% were positive at □300 ng/mL, 11.1% were positive at □2000 ng/mL, and 4.0% were positive for 6AM at □10 ng/mL. For opiate positive screens □300 and □2000 ng/mL, 91.3% and 80.8% confirmed positive for morphine or codeine at the respective GC/MS cutoffs. All positive 6AM screens confirmed by GC/MS. No specimens with opiates <2000 ng/mL confirmed positive for 6-AM. 6AM concentrations ranged from 200-9096 ng/mL. Increasing the opiate screening and confirmation cutoffs from 300 to 2000 ng/mL, resulted in 8% fewer opiate positive tests; however, recent heroin use (6-AM □10 ng/mL), was not affected by this change. Although the 6-AM screening assay identified fewer positive opiate tests, 4% compared to 11% with opiate □2000 ng/mL, it clearly identified heroin, as compared to poppy seed or codeine use.

CEDIA® is a registered trademark of Roche Diagnostics, Inc.

Keywords: 6-Acetylmorphine, opiate cutoff concentrations, CEDIA® DAU 6-AM, Heroin

The Effect of Ketoconazole on the Pharmacokinetics of l-α-Acetylmethadol (LAAM), and the LAAM Metabolites, norLAAM and dinorLAAM, in Opiate-Naive Subjects.

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l-α-Acetylmethadol (LAAM) is a mu opioid agonist approved as an alternative to methadone for maintenance therapy of opiate dependence. LAAM is extensively N-demethylated in humans by cytochrome P450 (P450) 3A4 to the more active metabolites, norLAAM and dinorLAAM. This production of active metabolites is believed to account for the prolonged therapeutic half-life that LAAM has in comparison to methadone. The involvement of P450 3A4 in LAAM metabolism suggests that this may be a site for drug interactions that could have a significant impact on the therapeutic action of LAAM.

To test this hypothesis, oral LAAM (5 mg/70 kg) was given to opiate-naive individuals in a cross-over fashion along with either ketoconazole (400 mg) or placebo administered orally 1 hour earlier. Blood and urine samples were collected and pupil diameters measured at regular intervals after drug administration. The data that follow are based on an N of 9 (4 males and 5 females). Ketoconazole had a significant effect on the in vivo metabolism of LAAM. This can be summarized as follows. The Cmax of LAAM was increased from 5.1 to 19.0 ng/mL, with an increase in plasma AUC of 44 to 261 ng/mL * hr. The Tmax (2 hr) and t1/2 (57-60 hr) were not affected. The Cmax of norLAAM was slightly reduced from 7.6 to 5.4 ng/mL while the Tmax was increased from 2.3 to 6.9 hr and the t1/2 was increased from 23.4 to 38.9 hr. The norLAAM AUC was increased from 116 to 277. The Cmax for dinorLAAM was decreased from 4.0 to 2.1 ng/mL while the Tmax was increased from 3.9 to 51.2 hr, but the t1/2 was essentially unaffected (40-48 hr). The dinorLAAM AUC was increased from 183 to 236. Changes in the twenty-four hour urine excretion amounts were similar to the plasma AUCs. Ketoconazole appears to inhibit both the N-demethylation of LAAM to norLAAM and the N-demethylation of norLAAM to dinorLAAM. This results in a delayed appearance of the active metabolites which was associated with a prolongation of the time to maximal pupil constriction from 7.8 to 25 hr. These results suggest that inhibitors of P450 3A4 may have an effect on the maintenance therapy of LAAM. (supported by NIDA grant R01 DA 10100).

Key Words: l-α-Acetylmethadol (LAAM) metabolism, Cytochrome P450 3A4; Drug Interaction

Oxycodone Associated Deaths in Southwestern Virginia

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Oxycodone is a semi-synthetic opioid analgesic available in both immediate and sustained release oral preparations. The frequency of oxycodone found in postmortem cases in southwestern Virginia is increasing. In 1998, the number of oxycodone cases totaled less than 10. In 1999 and 2000 the number increased to 19 and 30 cases, respectively. The increasing use and misuse of sustained release oxycodone for chronic pain has made interpretation of postmortem blood and tissue concentrations more complex.

We report a retrospective review of 46 postmortem oxycodone cases categorized by manner of death. A combination of ethanol and/or other drugs were found in 84% of cases. Ethanol and at least two drug classes were found in 16% of cases. Ethanol was found in 36% (18) of cases, narcotic analgesics 34% (17), benzodiazepines 54% (27), antidepressants 26% (13), antihistamines 20% (10) and 16% (8) miscellaneous drugs. There were 8 cases where oxycodone was the only drug found. In those 8 cases, 5 were accidental deaths and 3 were natural deaths. Analytical techniques used for confirmation and quantitation of oxycodone were GCMS and GC NPD.

Mean Oxycodone Blood and Liver Concentrations (mg/L or mg/Kg)

Category	N	Blood	Range	N	Liver	Range
Suicide	11	1.1	0.27 – 3.0	3	0.90	0.20 – 1.5
Accidental	25	0.41	0.06 – 1.2	2	0.41	0.32 – 0.50
Undetermined	3	0.31	0.20 – 0.44	0	n/a	n/a
Natural	7	0.23	0.07 – 0.51	1	0.52	n/a
Oxycodone Only	8	0.46	0.08 – 1.2	3	0.45	0.32 – 0.52

Key Words: oxycodone, postmortem, blood

mostly NE. U.S.

Blood [OC] 0.5 $\bar{x}$ (0.05 -2 ~~value~~)

← for accidental

accidental 0.1 ($\bar{x}$)

Codeine and Metabolite Disposition in Plasma and Saliva After Controlled Oral Codeine Administration

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Interest in monitoring drug exposure with saliva (SA), an alternative biological specimen, is increasing. The purpose of this study was to compare the disposition of codeine (COD) and metabolites in plasma (PL) and SA following oral COD administration. This IRB approved study was conducted on a closed research unit with informed consent provided by all subjects (n=19). Low (60mg/70kg) and high (120mg/70kg) doses of COD sulfate were administrered three times a week, four weeks apart. PL was collected for up to 48 h after first administration, and SA was collected simultaneously with citric acid candy stimulation. COD, norcodeine (NCOD), morphine (MOR) and normorphine (NMOR) were isolated by SPE followed by GC/EI-MS analysis. LOD's and LOQ's for all analytes were 2.5 ng/mL. COD was the primary analyte detected in both matrices. Initial detection in both PL and SA occurred within 0.08-1.0 h, and concentrations peaked within 0.5-4 h. Generally, a within-subject dose-concentration relationship was shown for COD disposition in PL (Cmax of 66-413 and 184-1158 ng/mL for low and high doses, respectively) and SA (Cmax of 184-1076 and 620-2757 ng/mL following low and high doses, respectively). Except during initial COD absorption/distribution, codeine SA/PL ratios were >1. Although there was considerable variability, mean COD SA/PL ratios were relatively constant from 0.5-12 h. Mean peak SA/PL ratios were 3.2 and 4.8 after low and high doses, respectively, except in one subject who had SA/PL <1. Salivary COD concentrations generally exceeded the proposed SAMHSA cutoff (40 ng/mL) from 0.5 up to 12 h. NCOD was detected in both matrices, but the primary COD urinary metabolite, MOR, and a minor metabolite, NMOR were not detected. From 0.5-12 h, NCOD/COD % ratios in PL and SA were 2-41 and 0.4-31%, respectively. These data demonstrate a similar COD detection window and metabolite profile for PL and SA following oral COD administration. Higher salivary COD concentrations coupled with the noninvasiveness, ease and lowered risk associated with SA collection make SA a viable alternative to PL for monitoring recent COD exposure.

Key Words: Saliva, Plasma, Codeine

The influence of sample site and collection technique on post-mortem morphine concentrations.

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In a series of 9 cases of death apparently due fatal misuse of illicit heroin we collected blood samples at post mortem from the jugular vein. A femoral vein was exposed in the iliac fossa and clamped. A sample was taken distal to the clamp and the clamp was then released and a free flowing proximal sample was collected. Finally, the leg was massaged from the ankle to up to the thigh and a further sample of blood was then collected from the femoral vein. The samples were assayed for total and free morphine concentration. The results are summarised in figures 1 and 2.

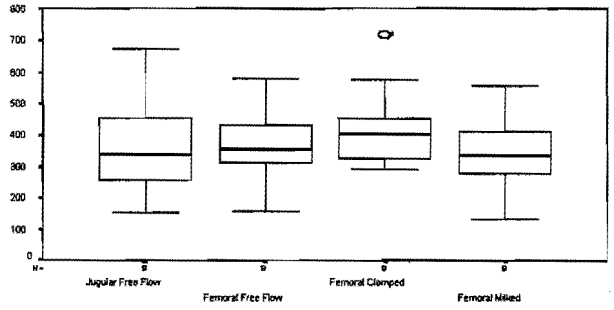

Fig 1. Total Morphine Concentration (µg/l)

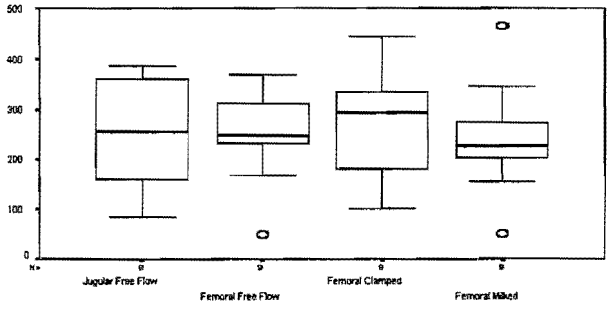

Fig. 2 Free Morphine Concentration (µg/l)

Whilst there were differences between the morphine concentrations found at different sites and using different collection techniques in individual cases, overall there was no significant difference in the concentrations of morphine from different sites collected by different techniques in this small series.

We conclude that the influence of site and collection techniques on post mortem morphine concentrations may have been overemphasised. Whilst they need to be taken into account, they probably are not the most significant factors in the interpretation of post mortem morphine concentrations.

Key Words; Heroin overdose, site dependency, blood collection technique.

Cr_2O_7 CrO_4

Analysis of Naltrexone and 6-ß-Naltrexol in Human Plasma by Liquid Chromatography - Electrospray Ionization Tandem Mass Spectrometry.

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Naltrexone (Trexan, Revia) is a pure mu opioid receptor antagonist used clinically for the treatment of opiate-induced toxicity. Because of its oral bioavailability and its long duration of action it can also be used as a therapeutic agent in the treatment of opiate addiction and alcohol dependence. Several methods have been published for the analysis of naltrexone and its primary metabolite 6-ß-naltrexol utilizing gas chromatography coupled to mass spectrometry. These methods, while providing excellent lower limits of quantitation (< 1 ng/mL), require extensive cleanup steps and chemical derivatization.

We present a sensitive and specific method for the direct analysis of naltrexone and its primary metabolite 6-ß-naltrexol in human plasma. Samples are fortified with deuterated isotopomers of both analytes and then made basic. The basified samples are then extracted with n-butyl chloride: acetonitrile (4:1). Extracted samples are chromatographed with reversed phase HPLC and ionized by electrospray ionization. Selected reaction monitoring MS/MS (*m/z* 342 → 324, naltrexone; *m/z* 344 → 326, 6-ß-naltrexol) is used to generate data for quantitation. This assay has a dynamic range of 0.1 ng/mL – 100 ng/mL for both analytes. Intra- and inter-assay precision had percent coefficients of variation less than 13% (intra) and 8% (inter) and accuracy between 95% and 108% of target concentrations at 0.1, 0.3, 5 and 75 ng/mL for both analytes. Analyte stability in plasma at 0.3 and 75 ng/mL was acceptable at room temperature for at least 24 hrs prior to extraction and after three freeze-thaw cycles. Prepared extracts at 0.3 and 75 ng/mL were stable for at least 48 hrs at room temperature and at least 5 days at –20 °C.

This work was supported by NIDA contract N01DA-7-8074.

Key words: Naltrexone, Plasma, HPLC-ESI-MS/MS

EtOAc solvent

4:1 n-Bu-Chloride/ACN —

good recovery, reasonable

selectivity for basic

compounds

Cocaine and Metabolite Disposition in Saliva and Plasma After Subcutaneous Cocaine Administration

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Healthy volunteers (n=19) provided informed consent to participate in this IRB approved study and resided on a closed research unit. Volunteers received 3 low dose (75 mg/70kg) subcutaneous injections of cocaine HCl on every other day, and after a 3-week interval, 3 high dose (150mg/70kg) injections. Saliva flow was stimulated using citric acid candy and collected using two types of Salivette™ (Newton, NC) swabs. Plasma was collected simultaneously for up to 48 h after the first dose. Samples were centrifuged and stored at –20 °C until analysis for cocaine and 12 metabolites by solid phase extraction followed by GC/MS. The limits of detection and quantitation for cocaine (COC) and benzoylecgonine (BE) were 2.5 ng/mL. COC was detected in saliva and plasma for 0.08–24 h; salivary Cmax occurred at 0.3–2.0 h and plasma Cmax slightly earlier at 0.1 – 1.0 h. Salivary Cmax ranged from 406–7116 ng/mL and 777–9260 ng/mL following the low and high doses, respectively. In plasma, Cmax concentrations were lower, 109–434 ng/mL after low dose and 253–1154 ng/mL after high dose. BE, the primary metabolite in both matrices, peaked within 4 h and remained detectable at 2.5 ng/mL for up to 48 h in most subjects. Salivary BE Cmax ranged from 70-440 ng/mL and 132-757 ng/mL following the low and high dose. Similar peak ranges were observed in plasma. A within-subject dose-concentration relationship for COC and BE was noted for both plasma and saliva. Saliva/Plasma COC ratios were >1 throughout the monitoring period, while BE ratios consistently were <1. According to the proposed SAMHSA oral fluid GC/MS confirmation cut-offs of 8 ng/mL for COC or BE, the mean ± SD detection times were 15.3 ± 8.0 h for COC and 29.2 ± 10.7 h for BE. These data demonstrate that saliva testing is a practical alternative to plasma testing due to the ease and lack of invasiveness of sample collection and difficulty of sample adulteration. Measurement of COC and BE in saliva is a valuable tool for monitoring recent cocaine exposure.

Key words: Saliva, Plasma, Cocaine

Abdominal Pain and Cocaine Use in an Inner City Emergency Department

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Abdominal pain associated with mesenteric ischemia has been reported with cocaine use, but the incidence of cocaine induced abdominal pain and its etiology has not been well described. We have previously reported heart failure, strokes and suicide gestures as common sequale of cocaine abuse. Abdominal pain is also a common chief complaint among patients with recent cocaine use presenting to an inner city emergency department (ED). We prospectively surveyed 1,988 patients with recent cocaine use. Cocaine use was determined by urine drug screen or patient report. Demographics and chief complaint as well as outcome were recorded. Among this series of cocaine users, 266 had a chief complaint of abdominal pain. The mean age was 37.7 ± 8 years, the majority were female (N=162, 61%). Most patients received an extensive work up for acute surgical causes of abdominal pain including electrolytes, comprehensive blood counts, liver function tests, amylase, ultrasound and/or CT scan of the abdomen. The workup in the majority of patients was normal (N=202, 76%). Seventy-six percent of patients were discharged from the ED (76%) with a diagnosis of abdominal pain of unknown etiology. Sixty four patients were admitted with diagnoses that included mesenteric ischemia, acute gastrointestinal hemorrhage, ketoacidosis, acute renal failure and perforated viscus. Among 53 patients where all medical records were reviewed, abdominal pain was a consistent complaint over time. Abdominal pain associated with cocaine is of unknown etiology. Better understanding of the etiology of abdominal pain related to cocaine use may lead to less costly workups.

Key Words: abdominal pain, cocaine abuse

A Drowning involving Ethanol, MDMA and Ketamine

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Recent interest in the recreational use of MDMA and ketamine has highlighted abuse in the rave scene. Ethanol and ketamine have also received publicity as drugs used to facilitate sexual assault. We present a case of a drowning involving the use of these three drugs.

A 25 year old white female was found in the Cuyahoga river after allegedly visiting a bar in Cleveland. Her body was recovered and transported to the Office of the Cuyahoga County Coroner for autopsy. Gross anatomic findings included thick white foam in the mouth and nose with froth in the airway and emphysema aqueosum. Microscopic examination revealed foci of over expansion and polarizable intracellular foreign material in the lungs. Specimens collected for toxicological analysis included heart and femoral blood, urine, cerebrospinal fluid (CSF) and vitreous humor (VH). Comprehensive toxicological testing was conducted as follows: volatiles; acetaminophen; salicylate; ethchlorvynol; immunoassay screen for illicit drugs in urine; acidic; neutral and basic drugs; and GHB. This testing was performed using headspace gas chromatography (GC); colorimetry; GC-FID; GC-NPD and gas chromatography/mass spectrometry. The table below illustrates the results:

	Heart Blood	Femoral Blood	Urine	CSF	VH
Ethanol (g/dL)	0.15	0.17	0.23	NTDN[1]	0.19
Ketamine (mg/L)	0.08	0.08	POS	0.08	QNS[2]
MDMA[3] (mg/L)	2.44	0.80	POS	0.50	QNS
MDA[4] (mg/L)	NEG	NEG	NEG	NEG	QNS
Pseudo/Ephedrine	NEG	NEG	POS	NEG	QNS

[1]NTDN= test not performed; [2]QNS= quantity not sufficient; [3]MDMA= 3,4-methylenedioxymethamphetamine; [4]MDA= 3,4-methylenedioxyamphetamine.

Investigation of the circumstances surrounding the death indicated that the deceased fell into the river while under the influence of drugs in the presence of a male companion. The Coroner ruled the cause of death, drowning and the manner, accidental.

Key Words: Drowning, MDMA, Ketamine

Comparison and Validation of Four ELISA Products for Detecting Cocaine in Hair.

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The objective of the study was to determine the optimum ELISA method for detecting cocaine in hair.

The analysis of hair for cocaine at a cutoff low enough to detect the majority of users is important to laboratories that perform analysis for drugs of abuse in hair. Comparison of four commercially available micro plate immunoassay products that are able to detect cocaine at low picogram per milligram levels is described. The amount of sample extract (pg/well) added to the wells of the plates was optimized for each product using the manufacturers recommended incubation times.

The linearity (R^2) for a plot of A/A_0 of points around the cutoff, and separation at –50% and +50% of the cutoff for each plate were calculated from this analysis. The results of the analysis are as follows:

Manufacturer #1: $R^2 = 0.859$ Separation = -35.4% and + 19.2%
Manufacturer #2: $R^2 = 0.740$ Separation = -8.5% and +11.2%
Manufacturer #3: $R^2 = 0.832$ Separation = -20.1% and + 14.1%
Manufacturer #4: $R^2 = 0.823$ Separation = -9.6% and + 45.9%

Conclusion: Manufacturer #1 was the most linear and had the highest degree of separation between the –50%, cutoff calibrator and +50% calibrator.

Key Words: ELISA, Cocaine, Hair Testing

Hair Exposed to Pyrolysis Products of Cocaine Freebase.

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Decontamination procedures are an important step in the analysis of hair. These procedures are designed to reduce concerns regarding external or passive drug contamination of the hair. The study reported here evaluated the effect of two wash procedures on the removal of cocaine and its metabolites deposited on rat and human hair. Drug-free rat hair (pigmented and non-pigmented) or human hair (black, red; bleached, nonbleached) was exposed to cocaine vapor by burning 1 mg cocaine freebase in a ventilated chamber. Hair was then washed with either: 1) isopropanol and dichloromethane, or 2) isopropanol and phosphate buffer, pH 5.5. Liquid chromatography/mass spectrometry was used to measure cocaine (COC) and its metabolites, benzoylecgonine (BE), ecgonine methyl ester (EME), and norcocaine (NORCOC). COC, BE, EME and NORCOC were detected in most non-washed hair. BE and EME were found at concentrations approximately 1% of the total COC and determined to be analytical artifact. Hair concentrations were significantly reduced compared to unwashed hair controls ($p \leq 0.05$) for some or all of the detectable analytes. The percentage of COC removed varied with the wash and hair type (93.0-100.0%), however in all but one instance neither wash removed all the drug. Although wash procedures appear highly effective at removing COC from the surface of the hair, measurable quantities (> 0.5 ng/mg) were still detected on most exposed hair.

Our study also evaluated the effect of the same two decontamination procedures when applied to hair from rats administered cocaine by intra-peritoneal injection. Again, the two washes significantly reduced (8.4-48.9%) COC concentrations compared to unwashed hair controls ($p \leq 0.05$) for some or all of the measurable analytes. The quantitative data obtained demonstrate that washing significantly removed environmental contamination or incorporated COC and its metabolites. However, drug was still detected after washing. Our results demonstrated that the limitations of each wash procedure should be identified during the validation of analytical methods. With this knowledge, laboratories should cautiously interpret quantitative hair data. This work was supported by NIH grant no. DA09096.

Key Words: hair, decontamination, cocaine

P-46

Postmortem Case Examples Involving Oxycontin®

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Oxycodone is a semi-synthetic opioid that is structurally similar to codeine and equipotent to morphine in producing analgesic effects. While oxycodone has been prescribed as many formulations (Percodan, Percocet, Tylox, Roxicodone, and Toxicet), the time release form of Oxycontin® has only been available since 1995. Oxycodone is prescribed in doses of 10-30 mg every four hours, whereas, Oxycontin® is prescribed in doses of 10-80 mg every twelve hours. In a two-year period, the Los Angeles County Department of Coroner's Toxicology Laboratory detected oxycodone in thirty-four cases, twelve of which were determined to be the time release form. The objective of this paper is to present information about Oxycontin® and to highlight case examples with unusually large numbers of undigested tablets in the stomach.

The isolation and identification of oxycodone from postmortem specimens was achieved via a basic, liquid-liquid extraction procedure with screening and quantitation by GC/NPD and GC/MS, respectively. D_3-oxycodone was utilized as an internal standard for quantitation with linearity achieved from 0.10 to 5.0 ug/mL.

The following table represents the tissue distribution ranges of Oxycontin® in the twelve case examples:

	Oxycodone (ug/ml or ug/g)						
	Heart Bld	Femoral Bld	Liver	Urine	Bile	Gastric (mg Total)	Vitreous
Range	0.12-46	+<0.10-5.5	0.11-1.9	3.2-~40	0.19-23	0.06-45	0.24-0.25
Average	4.6	1.3	0.91	15	6.0	12	0.25
Number	12	11	7	6	7	9 (8 cases with undigested tablets)	2

Keywords: Oxycontin®, Tissue distribution, Postmortem,

P-47

Identification and Quantitation of Morphine, Codeine and THC in Saliva using LC/MS/MS

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Target analysis of drugs of abuse in biological samples is of great importance for clinical and forensic toxicological investigations. Currently, plasma and urine are the most common matrices investigated. At present, GC-MS is the method of choice for confirmation of drug abuse. However, the procedure is labor intensive and time consuming particularly as derivatization is unavoidable. Tandem mass spectrometry utilizes specific molecular characteristics (molecular mass, fragmentation) for the detection of the compounds of interest.

Here we describe the development of a simple method for the quantification of three drugs of interest in saliva. We have investigated the mass spectrometric behaviour of morphine, codeine and cannabinoids with a view to developing a rapid LC/MS/MS analytical method that requires minimal sample pre-treatment, and allows for quantitation at the required levels of detection. Levels of recovery, precision and accuracy will be presented for spiked samples.

Keywords: Liquid chromatography/mass spectrometry, saliva, solid-phase extraction.

P-48

Toluene Artifact in Blood Collection Tubes

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Toluene was detected during headspace/gas chromatography (HS/GC) analyses for ethanol of DUI blood specimens collected in Corvac® brand red/gray stopper serum separator tubes. Parallel specimens collected in other types of collection tubes were devoid of toluene. During these routine analyses, toluene eluted in the next specimen in the run sequence. Accordingly, the method was modified for the specific quantification of toluene. Concentrations in 108 specimens collected in 31 different lots of Corvac® brand tubes were 4-74 mg/L. Blood collected in one lot contained no toluene.

The manufacturer has stated that toluene was used in the preparation of the separator plug. In order to measure the rate of diffusion of toluene into the collected blood specimen as well as the possible diffusion of other volatiles that might interfere with analyses for ethanol, blood from previously analyzed cases was added to two lots of blank Corvac® brand tubes provided by the manufacturer. These specimens were stored in typical fashion at 4°C and analyzed at various times thereafter. Specimens were selected from three cases containing no measurable volatiles, ethanol (0.287 g/dL) or acetone and 2-propanol (1360 and 2670 mg/dL, respectively). The blank blood specimen accumulated no measurable volatiles other than toluene. Ethanol diminished to 0.265 g/dL and acetone and 2-propanol diminished to 1050 mg/L and 2560 mg/L, respectively, all likely due to evaporation. Toluene accumulated to 15-21 mg/L within 21-53 days.

Because blood contained within this type of collection tube did not accumulate any other measurable volatile substance(s) that would interfere with the HS/GC ethanol analyses described herein, it was concluded that collection of blood specimens in Corvac® brand tubes poses no threat to the reliability of an evidentiary HS/GC analysis for ethanol provided the toluene does not co-elute with either the analyte or internal standard.

Key Words: Toluene, ethanol, headspace/gas chromatography

P-49

Simultaneous Determination of Opiates, Cocaine and Benzoylecgonine in Whole Blood and Urine Samples Using Polymeric Solid Phase Extraction and Gas Chromatography/Mass Spectrometry

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A rapid solid phase extraction (SPE) procedure was developed for the isolation and simultaneous identification of morphine, codeine, 6-monoacetyl morphine (6-AM), cocaine and benzoylecgonine (BE) in blood and urine samples. Drugs were isolated from one milliliter of blood or urine using new SPE cartridge with polymeric bonded phases (Cerex PolyChrom-Clin II™) that require no column preconditioning and minimal sample preparation. Deuterated internal standards, trimethylsilylation and selective ion monitoring were used throughout. Derivatized extracts were analyzed by GC/MS using a DB-5 capillary column and an HP 5973 MSD in the electron impact mode. Analytical performance was evaluated in terms of limit of detection, limit of quantitation, precision, accuracy, extraction efficiency, linearity and interferences. The following characteristic ions were utilized: morphine (429, 287, 324), codeine (371, 343, 234), 6-AM (399, 340, 287) cocaine (303, 182, 272) and BE (361, 240, 346).

The limits of detection for both cocaine and BE were 5 ng/mL in blood and 3 ng/mL in urine. The limit of quantitation for cocaine and BE in blood was 10 ng/mL. Extraction efficiencies for cocaine and BE were 94% and 66% in blood and 78% and 86% in urine. Intraassay precision was 1.7-4.2% (n=4; 100ng/mL) and accuracy was 89-94% (100 ng/mL). Using this method, extraction efficiencies for morphine, codeine and 6-AM were 86, 83 and 95% in urine and 50, 78 and 56% in blood. Limits of detection were 3 ng/mL for morphine and codeine and 2 ng/mL for 6-AM in urine and 5 ng/mL for morphine and codeine and 3 ng/mL for 6-AM in blood. Corresponding limits of quantitation in blood were 10 ng/mL for morphine and codeine and 5 ng/mL for 6-AM. Intraassay precision was in the range 1.5-7.2% (n=4) and accuracy was 94-111%. The procedure was modified to enhance the recovery of opiates from blood. Using the modified extraction, which was used only for opiate analysis, drug recoveries were increased to 60-85% for morphine, 73-100% for codeine and 67-73% for 6-AM. Evaluation of linearity, interferences and unknown samples were acceptable using either procedure. In conclusion, new polymeric SPE cartridges were used to isolate multiple drugs of interest from blood and urine with rapid and reliable results.

Key Words: Opiates, Cocaine, Solid Phase Extraction

P-50

Adulteration of samples donated for DAU screening: Not yet a problem in the UK?

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Over May 2001 our laboratory screened 1110 samples submitted for DAU screening for adulterants. The samples were an unselected mixture of clinical and workplace screening samples.

Many of the clinical samples were from patients with an incentive to provide negative samples, either because they are in a programme with a no drug use policy or because they are being treated with Naltrexone. The donors of work place screening samples have a clear incentive to provide apparently drug free samples.

Screening was carried out using EMIT ® reagents on an Olympus AU-600 analyser using standard protocols.

Screening for adulterants was carried out using Test True ™ reagents supplied Axiom Diagnostics Inc. The reagents used were for pH, nitrites, creatinine and chromates. In addition the samples were assayed for creatinine using the standard Olympus methodology used for clinical samples in our laboratory.

Setting up the assays was straightforward; the Axiom QA material provided yielded appropriate results

Less than 1.5% of the samples would have been rejected on DOH criteria. We found no samples that had an unphysiological pH, one sample that fulfilled the DOH criteria for "Substituted" and 13 that fulfilled the DOH criteria for "Dilute". One sample exceeded the cut off value for nitrite adulteration. None exceeded the cut off for chromate adulteration.

We were surprised with our results, particularly as the owner of a retail outlet in central Sheffield that sells both Zydot ™ and Klear ™ tells us that he has a modest but steady demand for these products.

We are grateful to of Axiom Diagnostics, for the donation of the Test True ™ reagents used in this study.

Key Words: DAU Screening, Adulterant detection, United Kingdom.

New EMIT® II Plus Cocaine Metabolite Assay with 150 ng/mL Cutoff

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A new Syva EMIT® II Plus Cocaine assay, for the cocaine metabolite benzoylecgonine, has been evaluated on the Syva 30R analyzer. The new method incorporates a new monoclonal antibody. Less than 5% overlap in rate measurement of controls at +/- 25% of a 150 ng/mL cutoff level was observed. In addition, less than 5% overlap was observed at +/- 25% of a 300 ng/mL cutoff level. In semi-quantitative mode, the recovery of controls in the range of 112.5 to 375 ng/mL benzoylecgonine was within +/- 20% of nominal values. No cross reactivity was detected for nineteen therapeutic and abused drugs non-structurally related to cocaine, including d-amphetamine and d-methamphetamine each at levels of 1000 µg/mL. There were no false positives in qualitative analysis of 1556 human urine samples at a 150 ng/mL cutoff, as shown below.

Cutoff of 150 ng/mL

		New EMIT® II Plus Assay -	New EMIT® II Plus Assay +
Current EMIT® II Plus Assay	-	**1416**	**0**
	+	**2◆**	**138**

◆Both samples failed to confirm as positive (< 100 ng/mL) by GC/MS.

The reagents are in liquid form. Samples were analyzed in both qualitative and semi-quantitative modes.

Key words: EMIT®, cocaine, benzoylecgonine

P-52

Development of a Rapid and Sensitive Method for the Quantitation of Amphetamines in Human Saliva.

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Target analysis of amphetamines in biological samples is of great importance for clinical and forensic toxicologists alike. Plasma and urine are currently the most common matrices investigated. However, the invasiveness of sample collection and opportunity for sample adulteration has led to an increasing interest in the use of saliva as an alternative matrix.

Currently GC-MS is the method of choice for confirmation of drug abuse. However, the procedure is labour-intensive and time-consuming particularly as solid phase extraction and derivatisation are unavoidable.

Here we describe the development of a simple, rapid HPLC-MS/MS method for the quantitation of amphetamines in saliva. Amphetamines were isolated from human saliva using a simple methanol extraction procedure and subsequently analysed using reversed phase HPLC-MS/MS using a Micromass Quattro *Ultima* triple quadrupole mass spectrometer. Quantification of the drugs and their deuterated analogues was performed using multiple reaction monitoring (MRM). The developed method, which has a chromatographic run time of less than 10 minutes, enables the simultaneous quantitation of several amphetamines in a single analysis. Limits of detection of 1ng/ml or better were achieved.

To assess the feasibility of using saliva as a non-invasive marker of drug abuse, the concentrations of six commonly abused amphetamines (MDMA, MDA, MDEA, amphetamine, methamphetamine and ephedrine) have been determined in saliva samples collected from current users. These values will be compared to corresponding levels in plasma and urine as determined by GC-MS.

Keywords: Electrospray Ionization (ESI); Forensic Chemistry; LC-MS/MS.

P-53

Fatality from Olanzapine Induced Hyperglycemia

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A case history of a 31 year old male schizophrenic patient is presented. The man was treated with olanzapine for 3 weeks before he died. After one week on a 10 mg daily dose of olanzapine, his fasting blood glucose was elevated to 11.3 mmol/L (203 mg/dL). In order to more aggressively treat his psychosis, the olanzapine dose was raised to 20 mg daily resulting in his fasting blood glucose climbing to 15.8 mmol/L (284 mg/dL). On the days preceding his death he became progressively weaker, and developed polydipsia with polyuria.

Post mortem blood, vitreous humor and urine glucose concentrations were 53 mmol/L (954 mg/dL), 49 mmol/L (882 mg/dL) and 329 mmol/L (5922 mg/dL) respectively. Drug screen on urine and blood indicated a small amount or olanzapine and no alcohols. Peripheral blood olanzapine concentration was within therapeutic limits, 45 ng/mL.
Vitreous and urine sodium, chloride, urea and creatinine support dehydration as the cause of death. Olanzapine was most likely causative for him developing diabetes mellitus. The extreme hyperglycemia which ensured, provoked a hyperosmolar diuresis which in turn caused dehydration, leading to coma and death.

Another atypical neuroleptic, clozapine has also been associated with the development of diabetes mellitus or diabetic ketoacidosis. We recommend including vitreous glucose and -hydroxybutyrate analysis as part of post mortem toxicology work up when the drug screen reveals the presence of any of either olanzapine or clozapine.

Keywords: olanzapine, clozapine, hyperglycemia,

P-54

Laboratory Analysis of Remotely Collected Oral Fluid Specimens for Methadone by Immunoassay

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The performance characteristics of a method for detecting methadone in oral fluid specimens were examined and compared with urine specimens. Oral fluid was obtained using a simple device that collects between 1 and 1.5 mL of fluid for laboratory analysis. When specimen or standard is added to an EIA well containing an oral fluid specimen positive for methadone, there is a competition between the drug and the enzyme-labeled hapten to bind the antibody fixed onto the EIA well. The EIA wells are then washed, substrate is added, and color is produced.

Specimens collected simultaneously from 102 methadone non-users and users from a drug treatment center were first tested using an immunoassay cutoff of 5 ng/mL in oral fluids and 300 ng/mL in urine. Using a second aliquot, methadone confirmation in urine was performed by GC/MS and in oral fluids by GC/MS. The combined immunoassay and GC/MS procedures were completed with less than 500 µL of oral fluid

The immunoassay was tested for precision, stability, and the effects of potential cross-reactants and interferences. The total precision for 20 days of testing calculated using the NCCLS EP5-T2 protocol yielded CV's less than 15%. The assay is specific for methadone but also has 18% cross-reactivity with LAAM. The assay exhibited no cross-reactivity to compounds such as acetylsalicylic acid, alprazolam, benzoylecgonine, caffeine, cotinine, d-amphetamine, ibuprofen, morphine, naproxen, penicillin, pseudoephedrine, and Δ^9-THC. The following adulterants did not interfere with the assay: sugar water, toothpaste, antacid, cola, and orange juice.

The results yielded 87.3% agreement between oral fluid and urine and 97.1% agreement between oral fluid and GC/MS, suggesting that oral fluid may be a reliable matrix for methadone detection.

Key Words: Methadone, Oral fluid, Saliva

P-55

Automated Sample Identification of Toxicological Samples Using Integrated Multi-Spectral Data

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For many years, scientists in the fields of forensics and toxicology have utilized gas chromatography/mass spectrometry (GC/MS) for the analysis of unknowns in samples. Large and thorough libraries containing spectra from electron ionization mass spectrometric (EI/MS) detection are available and provide convenient library searching capabilities. While GC/EI-MS is widely used and accepted, liquid chromatography (LC) with ultra-violet (UV) detection and atmospheric pressure ionization mass spectrometry (API/MS) is also gaining acceptance in the forensic and toxicology communities[1,2] due to its sensitivity and decreased requirement for sample derivatization (compared with GC/MS).

In the analysis of toxicological samples with LC/UV/MS, multiple types of spectral information (UV, EI-MS, ESI-MS) can be collected and exploited for the identification of unknowns. The integration of the spectral data generated from all of these detection methods into a searchable database has the potential to greatly assist in the analysis and throughput of samples. We will discuss the utility of a multi-spectral database for the identification of toxicological samples.

Toxicologically relevant compounds were isolated from various media (serum and salt solutions) and analyzed by UV, EI/MS, and ESI/MS. For the API/MS methods, multiple cone voltages were used to create characteristic fragment ions of the molecules by collision-induced dissociation (CID) on a single quadrupole mass spectrometer. The spectra generated by each detection method were entered into a searchable database. Once library spectra were generated, HPLC was used with the various detection modes to separate and identify the compounds.

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Key Words: LC/MS, library searching, multi-spectral

P-56

Determination of Ketosis in Previously Embalmed Autopsies using Vitreous.

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Recently, we encountered a case where starvation was suspected in a nursing home resident's death, but the body had been embalmed prior to autopsy. Acetoacetate (AcAc) reaches significant levels (10 mg/dL) in blood, serum or urine after 36 hours of fasting. Acetone is produced in much smaller quantities. However, embalming fluids contain formaldehyde which interferes with gas chromatographic (GC) detection of ketone bodies, and suitable blood and urine are not available from embalmed bodies. We sought to determine if the Acetest (Bayer Corp., Elkhart, IN), a simple, inexpensive, commercially available test for acetone and AcAc in body fluids would be usefule for the detection of ketosis in embalmed vitreous.

In our laboratory, acetone is routinely detected with headspace analysis by GC on a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) packed with Porapak S (Supelco, Bellafonte PA) porous polymer. Analysis is performed isothermally with oven and injector temperatures of 185°C and 195°C, respectively. Our method separates methanol, ethanol, acetone, 2-propanol and n-propanol, but not acetone from formaldehyde, with 2.85 and 2.65 minute retention times, respectively. GC analysis of vitreous from the case described and from a prepared control revealed a single peak at 2.75 minutes. Prior to analysis, AcAc was converted to acetone by heating up to one hour at 100°C. The nitroprusside based Acetest was performed by placing a drop of fluid on the test tablet, and a purple color formed if acetone or AcAc was present. We compared the Acetest to GC by testing vitreous samples known to be positive or negative for acetone or AcAc by GC. Sensitivity was determined by performing the Acetest on serial dilutions in pooled, non-ketotic vitreous with and without embalming fluid of a 50 mg/dL AcAc solution prepared from lithium AcAc (Sigma Chemical, St. Louis, MO) and deionized water. Final concentrations of AcAc ranged from 1.25 to 20 mg/dL.

Sensitivity was determined to be 10 mg/dL of AcAc. The Acetest was positive in all cases where GC was positive and the AcAc concentration was 10 mg/dL or higher. Significant interference from embalming fluid was not observed when typical concentrations were present. Concentrations of embalming fluid higher than encountered in practice caused false negative Acetest results. The autopsy case described was negative for ketones.

We conclude that the Acetest is a simple test that is useful for the detection of ketosis in embalmed specimens.

Keywords: vitreous, ketones, acetone, acetoacetate, starvation, ketoacidiosis, embalming

P-57

A Field Test of the Syva Rapid Cup: Implications for Use in Probation/Parole Supervision

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In the summer of 1999, the NH Dept. of Corrections initiated a field test of the Syva Rapid Cup (5-drug panel) to determine if the Probation and Parole Division would adopt this type of drug testing procedure. Over an eight-month period, 13 field officers collected 802 urine samples from probationers and parolees. The results were read and recorded by the officers, but no action was taken. All samples, both positive and negative, were sent to the central NYDOC lab where they were initially screened using and Emit 30R. all positive screens along with any discrepant readings by the officers were then tested with the Gas Chromatopraphy/Mass Spectrometer (GC/MS), considered the gold standard for accuracy. In addition, pre and post-tests were developed for the 13 Field Services staff to measure satisfaction with the cups. Test results showed that Positive Predictive Values for THC, Opiates, and Cocaine were .66, .64 and .88 respectively. The accuracy of the cups (TP + TN/Total Samples) as compared to the GC/MS, ranged from a low of 89% for THC to a high of 95% for cocaine. A total of 101 false positives (13%) and 12 false negatives (1.5%) were found among the 802 cups. Forty urine samples were found to be positive for drugs for which the cups do not test and 47 other samples were found to be diluted, unable to be detected by the cup. The cup failure rate (unreadable for at least one drug) was 5%. Out of the total sample, 241 cups (30%) presented problems that could be of significant concern to a Probation/Parole organization, possibly leading to false imprisonment, litigation and failure to detect to total range of drug use among its supervisees. Furthermore, the results of the pre and post-test demonstrated a diminished desire to use the cups among the field staff (t-2.803,11df, $p < .017$) as well as a decreased in other desirability facotrs following the eight months of use. Policy and treatment decisions based on the use of the cups may need to be revisited given the limitations and shortcomings of this method of urine drug testing

P-58

Estimation of uncertainty in blood alcohol analysis

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Many conclusions in forensic chemistry as well as in other analytical laboratories are based on the results of chemical quantitative analysis. Whenever decisions are based on analytical results, it is important to have some indication of the quality of the results, that is, to the extent to which they can be relied on for the purpose in hand.

The evaluation of uncertainty requires the analyst to look closely at all the possible sources of uncertainty. However, although a detailed study of this kind may require a considerable effort, it is essential that the effort expanded should not be disproportionate.

Methods and Instrumentation: Blood alcohol analysis is performed by Headspace Gas Chromatography in duplicate on two different columns. Calibration curves are made bi-monthly in connection with maintenance. Control samples are prepared for each series of samples. Five PerkinElmer Autosystem GC with HS 40 samplers are used in connection with TurboChrom Client/Server and a dedicated Laboratory Information Management System

Results: The relationship between the measured values (y) end the expected values (x) of control samples could be described by following function:

$$y = \alpha * x^{\beta_1}$$

given the possibility to construct an control card based on the equation:

$$\beta_1 = \log(y) - \log(x) = \beta_0 + e_i$$

The measurement uncertainty is calculated using group wise combination of the identified uncertainty components.

Key words: Blood alcohol, Headspace-Gaschromatography, Uncertainty budget.

P-59

Utility of LSD screening of forensic urine specimens

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According to the summary of findings from the 1999 National Household Survey on Drug Abuse (DHHS) there were 900,000 current hallucinogen users. The majority of laboratories conducting medicolegal death investigations in the United States do not routinely screen specimens for LSD. LSD is rarely implicated as a cause of death. In the laboratory of the Cuyahoga County Coroner, postmortem specimens, DUID and probation (criminal justice) specimens are tested. The objective of this study was to assay a representative subset of specimens for LSD to determine if routine LSD screening was warranted.

A set of 442 urine specimens from routine cases were screened for LSD using the STC Technologies, Inc. (Bethlehem, PA) LSD Micro-Plate EIA. Each specimen was assayed in duplicate according to the manufacturer's instructions. Results were evaluated using both a 0.25 and a 0.50 ng/mL cutoff. Using the lower cutoff concentration, 43 specimens were positive, and using the higher cutoff only 17 specimens were positive. At the lower cutoff concentration, 63% of the screening positive specimens were from post mortem cases. All presumptive positive specimens were confirmed by solid phase extraction followed by LC/MS/MS using a Micromass Quatro II instrument. Specimens were assayed for LSD, and the metabolites Nor-LSD and Iso-LSD. The calibration curve utilized 6 standards at concentrations ranging from 0.05-10.0 ng/mL. The limit of detection for each analyte was 0.025 ng/mL and the limit of quantitation was 0.05 ng/mL. LSD and metabolites were not confirmed in any of the 43 presumptive positive specimens.

The authors concluded that LSD screening in their sample population was not warranted. Even though 9.7% of specimens screened positive for LSD, LSD use was not confirmed. The screening assay may have detected analytes not targeted in the confirmation test since LSD is extensively metabolized in humans. Alternatively, other urine constituents may have been responsible for the false positive results. Since screening positive results occurred in clinical and postmortem specimens, the results were unlikely to be due to a postmortem artifact. This work illustrated the utility of conducting a pilot study before spending valuable resources initiating a full testing program with limited benefit.

The authors thank Orasure Technologies, Inc. (formerly STC) and National Medical Services, Inc. (Dr Michael Robertson) for conducting the urine drug testing.

Key Words: LSD, micro-plate EIA, LC/MS/MS

P-60

Assessment of the utility of Opiate hydrolysis in the number of positive urine specimens in a Criminal Justice drug testing program.

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The aim of a forensic urine drug testing program in the criminal justice system is to monitor compliance with a non drug use policy. Detection and deterrence of illicit drug use is the primary goal. Therefore, it is desirable to detect 6-acetylmorphine (6-AM) in the cases of a heroin user rather than morphine which may be attributed to alternate sources. The practical result of such a policy is to assay specimens without hydrolysis as a sample preparation step in the procedure. An acceptable result of this policy is not detecting some positive samples since in the majority of cases it is not feasible to assay each specimen twice. The objective of this study was to assay urine specimens hydrolyzed and unhydrolyzed in order to assess the potential increase in the number of opiate positive specimens compared with the loss of 6-AM detection.

Urine specimens were hydrolyzed after phosphate buffer addition (pH 6) with β-glucuronidase Type H-2 (helix pomatia) at 35°C. A 500 ng/mL morphine 3-glucuronide hydrolysis control was assayed concurrently with case specimens. Hydrolyzed and unhydrolyzed specimens were subject to SPE GC/MS analysis using a four point calibration (20-400 ng/mL) and nalorphine as internal standard. Deionized water and sodium phosphate buffer were added to samples before addition to Clean Screen® extraction columns. After washing the columns with deionized water, acetate buffer (pH 4.5) and methanol, samples were eluted with methylene chloride:isopropanol:ammonium hydroxide (80:20:2). Samples were assayed for opiates on an HP 6890 GC interfaced with an HP 5973 MSD using a DB-5 capillary column (J&W Scientific). The lowest reporting limit was 5.0 ng/mL for 6-AM, 40 ng/mL for oxycodone and 10 ng/mL for morphine, codeine, hydrocodone and hydromorphone.

Of 112 urine specimens, 6 specimens were positive after hydrolysis that had been negative when assayed unhydrolyzed. In all cases the drug detected was morphine. Morphine was detected in 4/6 specimens when assayed unhydrolyzed but was present below the reporting limit (4-9 ng/mL). Furthermore, one specimen contained 6-AM which was not detected after hydrolysis. Morphine concentrations in the 2 remaining positive cases were 12 and 43 ng/mL after hydrolysis with no detectable morphine present when assayed unhydrolyzed. The authors concluded that since hydrolysis resulted in an increase in the number of positive specimens of only 5.3% and resulted in the loss of 6-AM, routine hydrolysis was not cost effective.

Key Words: Urine drug testing, Opiates, Hydrolysis

P-61

Validated High-Throughput Determination of Drugs of Abuse for Employment and Judicial Evidence by Fast-Temperature Programming GC

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Since the first Turkish nomads smoked the resin gum of the Opium poppy and the first Incan priests chewed Coca leaves; Drugs of Abuse (DOA) in world culture became a standard. Most industrial and retail employment requires drug screening prior to acceptance. The legal system uses DOA testing for evidence in Driving Under the Influence and insurance cases. The most universally recognized analytical protocol for such testing to provide evidentiary material, based on the methods of DEA, FBI and USP/NF; is Gas Chromatography-Mass Spectrometry (GC-MS).

Having the ability to determine any alkaloid, barbiturate, amphetamine or new designer drug with the multi-dimensional capacity of GC-MS is extremely valuable. Sample preparation has been greatly simplified and automated, many samples can be extracted by continuous-flow solid phase extraction (SPE) or on-line solid phase microextraction (SPME) rapidly. The slow link in the cycle being the actual analysis: the chromatographic process. Using the classic, oven-heated GC system, a typical run-time from injection to injection can be 8-10 minutes. Employing the newer design of the EZ-Flash direct thermal heater with an MS-grade capillary column, such analytical cycles can be reduced to less than 3 minutes with complete preservation of resolution and peak integrity. This presentation will focus on the determination of delta-9-Tetra-Hydro-Cannabinol (9-THC) and its metabolites, as one of the more "popular" components. Using the EZ-Flash GC separation with SIM analysis, and following all required protocols of existing methods; the determination of these 5 ions [542/693 from the Internal Standard, 477/539/690 from the 9-THC] provides confirmatory data accepted by the judicial system.

Key Words: Gas Chromatography-Mass Spectrometry (GC/MS), Fast GC, Drugs of Abuse

nice idea, but cost is $20K! - would be better to buy a GC equipped w/ fast GC - also, possible problems w/ software - may require upgrade.

Pharmacogenomics for Forensic Pathology-Toxicology:Genotyping CYP450 2D6 as an adjunct for certifying methadone toxicity

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Pharmacogenomics may be used as an adjunct for certifying drug toxicity. In particular, the identification of a mutation in the CYP450 enzyme 2D6 (*3, *4, and *5) may be used as an adjunct for certifying the cause of methadone deaths. Methadone is metabolized by the CYP450 3A4 and 2D6. CYP450 2D6 is polymorphic. Prevalence of poor metabolizers ranges between 5 - 10% in the general population and 95% of these subjects are homozygous for *3, or *4, or *5, or heterozygous for any two of these mutant alleles. The objectives of this study are: firstly, to perform a retrospective study of methadone cases between 1998 and 2000 and secondly, to genotype CYP450 2D6 of selected cases by conventional and Light Cycler-Real Time PCRs. This retrospective study showed 39 of 282 opioid cases were positive for methadone. 21 of these cases (54% averaged over the study period) were certified with methadone toxicity as the cause of death. Decedents were predominantly male (77%) with the average and range of methadone concentrations being 0.49 and 0.10-2.4 mg/L. In addition, 62% of the methadone related deaths occurred between Friday and Monday, with a high of 75% in 1998. The most frequently co-ingested drugs were opiates, benzodiazepines and ethanol with maximal frequencies up to 50%, 53% and 60% respectively. Genotyping showed 1 heterozygous for 2D6*3, 2 homozygous for 2D6*4, 5 heterozygous for 2D6*4, and 1 heterozygous for both 2D6*3 and *4. By stratifying the genotyping results with methadone concentrations, case history, autopsy and other toxicological findings, 2D6 mutations for 4 of the above 9 cases may have resulted in the elevated methadone concentrations. Thus, genotyping may serve as an adjunct for certifying methadone toxicity.

Key: Pharmacogenomics, CYP 2D6, methadone toxicity

details of procedure available

Prevalence of Methamphetamine, Amphetamine, MDMA and MDA in Human Hair

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Over 100,000 hair specimens were screened for amphetamines using a micro-titer plate enzyme immunoassay (International Diagnostic Systems Corp.) with high cross-reactivity to methylenedioxymethamphetamine (MDMA) from August of 2000 to May of 2001. The majority of the specimens analyzed were from the employment testing population. The immunoassay was evaluated by receiver operating characteristic (ROC) analysis to determine optimum analytical cut-off values in hair specimens. Utilizing 300 pg/mg of d-methamphetamine as the cut-off value, the sensitivity and selectivity of the assay was 86% and 100%, respectively. Samples which screened positive were confirmed by gas chromatography / mass spectrometry (GC/MS) for amphetamine, methamphetamine, MDMA and MDA. Over 3,000 specimens contained some combination of methamphetamine, amphetamine, MDMA, and MDA. Methamphetamine was confirmed most often, followed by amphetamine, MDMA and MDA, in samples that screened positive for amphetamines. Over 700 of the amphetamine positive samples contained MDMA.

Key words: hair, methamphetamine, MDMA

APL

SCREEN AMP hair Elisa

300 pg/mg cutoff

wash w/ MeOH
2-3 mL MeOH 75° 2hr
add acid
dry down
analyze

most [MA] hair ≈ 1 ng/mg
most [MDMA] hair ≈ .05 ng/mg

Urinary Methamphetamine and Amphetamine Detection Times Following Controlled Oral Administration of Methamphetamine.

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Methamphetamine (MAMP) is targeted in workplace, military and forensic urine drug testing due to its adverse performance effects, abuse liability and the medical sequelae resulting from abuse. Current SAMHSA requirements for a positive MAMP workplace urine confirmation include a MAMP GC/MS ≥500 ng/mL and an AMP ≥200 ng/mL (500/200). SAMHSA has recently proposed new confirmation cutoffs of 250 and 100 ng/mL for MAMP and AMP, respectively (250/100). The goal of this study was to evaluate urine confirmation cutoffs in determining MAMP exposure. The study was IRB approved, conducted on a closed research unit, and subjects provided informed consent. Four daily 10 mg (n=8) and 20 mg (n=5) oral doses of (d)-MAMP HCl were administered, and urine specimens were collected ad libitum prior to and for two weeks following drug administration. MAMP and AMP were isolated by SPE, and measured by GC/PCI-MS. No intra- or inter-subject dose-concentration relationship was found with creatinine normalized or non-normalized data. Employing assay LOQ's (2.5 ng/mL), MAMP (82-1827 ng/mL) and AMP (12-179 ng/mL) were generally detected in the first post-administration void. MAMP ≥500 ng/mL occurred in the 1st or 2nd void (1-11 h) at concentrations of 524-1871 ng/mL. Lowering the MAMP cutoff resulted in little or no change in initial detection times. AMP ≥200 ng/mL occurred in the 2nd-13th (7-20 h) void and was generally delayed with shorter micturition intervals. Lowering the AMP cutoff to 100 ng/mL shifted initial confirmation to the 2nd-8th void (4-13 h). Employing a 250/100 cutoff extended MAMP detection 2-24 h (1-7 voids), and total detection time increased by 2-34 h (1-10 voids). Lowering the cutoff also increased the number of positives identified from first to last positive by as much as 33%. Inter-subject variability in AMP/MAMP ratios impacted detection times and suggested differences in MAMP metabolism. These data indicate that lowering workplace urinary cutoffs to 250/100 will shorten initial detection times, extend detection windows and increase confirmation rates for MAMP exposure. The AMP requirement for a positive MAMP urine test was instituted to further analytically substantiate MAMP exposure; however, elimination or reduction of this requirement would improve MAMP detection times and confirmation rates.

Keywords: Methamphetamine, Urinalysis, Confirmation Detection Times

proposed ↓ cutoffs from 500/200 → 250/100

following dose of MA, [A] > [MA] @ ~4 days

implication dropping the necessity of 200 Amp in presence of 500 Meth caused significantly ↑ % positives, detection window

Analysis of Methylenedioxymethamphetamine and its Analogs in Urine by Liquid Chromatography - Electrospray Ionization Mass Spectrometry.

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The use of methylenedioxymethamphetamine (MDMA) is becoming an increasingly important problem for the forensic toxicology community. The drug's popularity at "rave" parties makes its use a particular concern with younger people. In order to address the issue of MDMA use forensically, analytical methods that are specific for MDMA and its congeners are needed. High performance liquid chromatography coupled to atmospheric pressure ionization mass spectrometry (API-MS) is a useful technique for the direct analysis of low concentrations of small organic molecules such as MDMA and other abused drugs.

We present a sensitive and specific method for the analysis of MDMA, methylenedioxyamphetamine (MDA) (the principle metabolite of MDMA), and methylenedioxyethylamphetamine (MDEA) (a structural analog of MDMA) in urine. Urine is fortified with a deuterated isotopomer of MDMA and then made basic. The basified samples are extracted with n-butyl chloride. Extracted samples are chromatographed with reversed phase HPLC and ionized by electrospray ionization. Selected ion monitoring is used to generate data for quantitation. This method has a dynamic range of 25 ng/mL to 1000 ng/mL. Inter- and intra-assay precision had percent coefficients of variation less than 15% and accuracy was within 5% of target concentrations for MDMA at 100 and 500 ng/mL. In the past 2 years, 276 urine samples have been submitted to the laboratory for MDMA analysis. In each case reported as positive, both MDMA and MDA were identified. No samples tested have been positive for MDEA.

Key words: MDMA, Urine, ESI-LC-MS

Fatal Gasoline Poisoning: Detection and Quantitation of Petroleum Hydrocarbons by Gas Chromatography/Mass Spectroscopy

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GC-MS was used for identification of petroleum hydrocarbon patterns and quantitation of selected hydrocarbons in blood and body tissues for the investigation of a human fatality involving suspected gasoline inhalation overdose. Preservation of volatile hydrocarbons in blood and body tissue samples was maintained by collection in crimp-sealed vials at autopsy and refrigerated storage. Ambient temperature headspace samples from blood and tissue were analyzed by capillary-column (dimethyl polysiloxane) gas chromatography interfaced with an ion-trap mass spectrometer using electron impact ionization. The method was validated for the detection of 18 hydrocarbons found in gasoline.

Analysis of liquid petroleum product in a container found with the decedent demonstrated an organic volatile pattern consistent with petroleum hydrocarbons found in gasoline, and the pattern in postmortem blood, brain, kidney and liver was consistent with gasoline intoxication. Postmortem blood concentration (mg/L) of heptane (1.86), toluene (6.02), octane (1.94), p-xylene (5.02), and o-xylene (1.73) were determined by a method employing primary standards and use of 1-chlorobutane and 1-chloropentane as internal standards. The level of hydrocarbons in brain, kidney and liver exceeded blood, with liver tissue contained the highest concentration (mg/kg) of heptane (88.7), toluene (282), octane (100), p-xylene (68.3), and o-xylene (34.9).

The estimation of total gasoline concentration in blood and tissue based upon individual hydrocarbon levels was variable due both to differences in pharmacokinetic distribution and elimination of individual hydrocarbons in the body and to varying proportions of hydrocarbons in commercially available gasoline. Direct quantitation of a panel of prominent hydrocarbons is recommended for evaluating and comparing exposure in cases of gasoline intoxication. The case demonstrates the application of qualitative and quantitative headspace GC-MS analysis of volatile hydrocarbons in a death investigation involving gasoline.

Key Words: Gasoline, fatality, headspace GC/MS

Postmortem Tissue Concentrations of Risperidone

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Risperidone (Risperdal®, Janssen Pharmaceutical), a benzisoxazole derivative, is an atypical antipsychotic with strong binding affinity for serotonin 5-HT_2 and dopamine-D2 receptors, as well as a high affinity for α_1- and α_2-adrenergic and histamine H1 receptors. It is designed to treat both positive (hallucinations, delusions, and thought disorders) and negative (emotional withdrawal, blunted effect, and loss of speech) symptoms of schizophrenia. 9-hydroxy-risperidone, the formation of which is mediated by cytochrome P450 2D6, has a much longer half-life ($t_{1/2}$= 20.5 h versus 2.8 h for risperidone in extensive metabolizers).

Risperidone and 9-hydroxy-risperidone were identified by liquid chromatography-mass spectrometry (LC-MS) using atmospheric pressure ionization (API) electrospray in positive mode following an n-butyl chloride extraction. In each of seven cases where risperidone was detected, the specific distribution of both compounds is reported. Postmortem tissue concentrations of risperidone and 9-hydroxy-risperidone ranged from <0.02-1.8 and <0.02-6.2 mg/L, respectively, and were generally higher in bile and urine and lower in blood and vitreous fluid. In one case where both central and peripheral blood had been collected, there was a marked difference in concentration (0.3 versus 1.8 mg/L in peripheral and central blood, respectively). Risperidone was typically present with other drugs, including other antidepressants, alcohol, benzodiazepines, and opiates. As many antidepressants and some opiates are also substrates and/or inhibitors for CYP 2D6, the potential for competitive inhibition of metabolism exists. Additionally, risperidone has the potential to interact with other serotonergic drugs, and may contribute to the onset of serotonin syndrome. The specific mechanisms of interaction, as reported in the literature, are reviewed and evaluated in the investigation of seven fatalities occurring over an eighteen-month period. Five cases were classed as overdoses of one or more other drugs present. Over the eighteen-month period of this study, blood concentrations exceeded the therapeutic range for risperidone in only one case. There were no deaths ascribed solely to risperidone intoxication.

Key Words: Risperidone, LC-MS, Postmortem Toxicology

OTR < 10ng/mL - almost necessitates LC/MS

EKTN: ↑pH, n-BuCl

Buch ext: 0.2% H_3PO_4

toxic/elevated levels very high ≥300 ng/mL

ERA-1

Characterization of [^{3}H]-Flunitrazepam Binding to Melanin

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In both clinical and forensic toxicology, the analysis of hair for drugs is an important tool to determine drug use in the past or to verify abstinence from illegal drugs during extended periods. Melanin is proposed as one of the factors that influence drug incorporation to hair and we have characterized the binding of the drug flunitrazepam to melanin *in vitro*.

Tritiated flunitrazepam and melanin granules from cuttle fish, *Sepia officinalis*, were used. Measurements were done in a beta-counter directly on the melanin after filtration.

The results showed a rapid binding followed by, not saturation, but an almost linear slope of slowly increasing drug binding. A solely electrostatic attraction to the surface would decrease the more drug that bind until saturation. The data fitted excellently to a curve composed of one term containing the square root of time added to one Langmuir binding term. Initially, the Langmuir dominating binding may reflect a superficial binding to the surface of the melanin granule. This is followed by a binding limited by diffusion as is suggested by the fit to the square root of time. We believe that this binding reflects the diffusion of drug molecules into the matrix of melanin deeper in the granule. We chose to call these *surface binding* and *bulk binding*, respectively.

The binding of a group of benzodiazepines and tranquilizers was compared to the binding of [^{3}H]-flunitrazepam by means of displacement experiments. This showed similar binding characteristics except for phenobarbital, which had a lower affinity.

The method developed in this study allowed measurements with low melanin and drug concentrations, and had the strength to propose binding characteristics for flunitrazepam to melanin as well as measure the relative binding of several other tranquilizers.

Keywords: Benzodiazepines, melanin, hair

ERA-2

Urinary Excretion of Δ9-Tetrahydrocannabinol (THC), 11-Hydroxy-THC (11-OH-THC), and 11-Nor-9-Carboxy-THC (THCCOOH) in Long Term Marijuana Users

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Limited quantitative data are available on the timecourse of excretion of inhaled cannabinoids into urine. Kemp et al (J Anal Tox 1995) first suggested that hydrolysis with E. coli beta-glucuronidase was necessary to hydrolyze THC- and 11-OH-THC glucuronide in urine. This study examined the excretion timecourse of THC, 11-OH-THC, and THCCOOH in urine specimens (N=430) of 18 male and female long-term marijuana users participating in a neuropsychological performance study. They had smoked marijuana on at least 5000 occasions prior to entering the 28 day outpatient abstinence study. Specimens were collected daily under direct supervision until <20ng/mL for two days; followed by every other day collection. Specimens were frozen at -20°C. Sample preparation included enzymatic hydrolysis with E. coli beta-glucuronidase, alkaline hydrolysis, solid phase extraction (UCT CleanThru DAU columns), BSTFA derivatization and GC/MS analysis (THC, 11-OH-THC LOQ of 1 ng/mL, THCCOOH LOQ of 2.5 ng/mL). THCCOOH was ∃15 ng/mL for six subjects over 28 days. Creatinine normalized THC, 11-OH-THC, and THCCOOH were evaluated to monitor new drug use. Preliminary data for 18 of 74 subjects indicated that THC, and 11-OH-THC was excreted over a much longer timecourse in long term marijuana users than previously reported following acute exposure. Maximum concentrations, mean ng/mL ± SD (range) were 19.2 ± 16.2 (0.0-69.7), 85.5 ± 111.5 (10.4-463.8), and 307.1 ± 395.5 (29.9-1612.1), respectively for THC, 11-OH-THC, and THCCOOH. Also, 11-OH-THC appears to increase earlier than THCCOOH after smoking. Additional excretion data collected in a controlled research environment are needed to more accurately interpret new drug use in long term marijuana smokers.

Key Words: THC, metabolites, smoked marijuana, GC/MS, urine excretion

Substance Abuse and In-Custody Deaths

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In custody deaths represent a potentially costly legal forum. Despite common accusations of improper care, the majority of in custody deaths are not unexpected and are not a result of improper care. We retrospectively reviewed all in custody deaths from 1995-2000 for the local law enforcement agency. In this 6 year period, 24 inmates died while in jail. All were male, the mean age was 44 ±9 years with an age rage of 22-64 years. Of the 24 inmates who died in custody, 21 had a history of substance abuse, most notably alcohol (19/21). Of the 19 with alcohol abuse, nearly half had end stage liver disease and/or hepatitis Cocaine use was documented in 6 patients, opioid use in 3. Twenty one deaths were ruled as natural, three were of unknown cause. All 24 patients had prior admissions to the hospital and were discharged back to jail. In the 24 cases we reviewed, all but 3 deaths are attributable to substance abuse, most notably alcohol and/or cocaine. The most common mechanisms of death is sudden cardiac death, presumably from arrhythmias. Other modes of death include seizures and cerebral vascular accidents. It is important to develop a history of substance abuse for in custody death cases.

Key Words: in custody death, substance abuse, sudden death

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Postmortem Drug Redistribution

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One of the most difficult responsibilities of the forensic toxicologist is the interpretation of postmortem drug levels and their possible significance as to behavior and/or cause of death. During the past 15 years much work has been performed using human case information and animal studies to illustrate and validate the phenomena of postmortem drug redistribution. These studies provide certain clarification to drug level interpretation. They also cast uncertainty on the interpretation of drugs where analogous studies are incomplete or totally absent. Literature data of more than thirty [30] drugs, as reported by numerous authors, are reviewed to illustrate two phenomena: (a) postmortem redistribution occurs primarily as a function of diffusion of drugs along a concentration gradient, from sites of high concentration in solid organs into the blood with artificial elevation of the drug levels; (b) while many drugs seem subject to postmortem redistribution, there are also others that undergo no change whatsoever. While additional work still needs to be performed, there has been enough achieved to both illustrate and validate that postmortem redistribution does exist. These informations are helpful to forensic pathologists in determining sample set taken during autopsy.

KeyWords: Central blood, peripheral blood, postmortem drug redistribution, solid organ diffusion.

Death by Paint Thinner

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A 38 year-old Caucasian male, reportedly missing for four days, was found dead forty feet down a steep ravine, apparently after jumping down the cliff. He had shown depressive symptoms over a recent divorce and had been admitted for suicidal ideations four months before, with continuous psychiatric consultations since. He reportedly received Haldol® , Lithium, Zyprexa®, and Dilantin® for his condition.

He was found with lividity and rigor consistent with his position. An odor of a petroleum distillate was noted about his body. Abrasions and rock indentations were noted on the right shoulder, both forearms and knees. Loose skin was observed on the back and buttocks with no defensive wounds observed. In his vehicle, found at the top of the cliff, were two rectangular cans, one quart and one gallon size, of paint thinner.

The autopsy report indicated that the decedent was normal with a few exceptions. The heart was large (510g) and myocardium was soft and congested. The lung parenchyma reveals congested, atelectatic, edematous and hemorrhagic lungs with multiple patches of bronchopneumonia and a higher than normal lung weight (1350g). Multiple stress ulcers thoughout the gastric mucosa with the esophageal mucosa showing discoloration. Stomach contents revealed 150 mL of brownish liquid with a nearly clear oily thick film with the small and large bowels showing oily liquid with a strong odor of a petroleum distillate.

Toxicological analysis was negative for ethanol and common drugs of abuse. Found in therapeutic concentrations were valproic acid, diphenhydramine, and norsertraline and in sub-therapeutic levels were sertraline, diazepam, and nordiazepam. Bupropion metabolites were detected.

Static adsorption-elution, commonly used in fire debris analysis, was used to examine the brain, liver, lung, blood, and urine. A liquid-liquid extraction was performed on the vitreous humor. The stomach contents and samples from the cans were diluted with carbon disulfide. All but the blood and vitreous contained a medium petroleum distillate. The stomach content was consistent with the liquid from the one gallon can. Chromatograms suggest differential metabolism and/or distribution among the different organs.

Keywords: Paint thinner, suicide, pulmonary edema

Deposition of 7-Aminoclonazepam and Clonazepam in Hair After a Single Dose of Klonopin™

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The objective of this paper was to determine whether benzodiazepine clonazepam (CLO) and its major metabolite 7-aminoclonazepam (7ACLO) could be detected in hair collected from 10 heathy volunteers after receiving a single 3 mg dose of Klonopin™ (CLO). Such data would be of great importance to law enforcement agencies trying to determine the best time interval for hair collection from a victim of drug-facilitated sexual assault in order to reveal drug use. To achieve the above goal, higly sensitive NCI-GC-MS method for the simultaneous quantitation of CLO and its major metabolite 7ACLO in hair was developed and validated. Hair samples collected from volunteers who received a single dose of CLO were then analyzed. Ten healthy volunteers (6 women and 4 men, 23-49 years old) participated in the study. The following hair samples were collected from each volunteer: one before CLO administration, and 1, 3, 5, 14, 21 and 28 days after. All hair samples were pulverized and 50 mg aliquots were sonicated in methanol and digested with 0.1 N HCl at 55^0 C for 18-24 hours. Internal standard, D_5 diazepam (600 pg/mg) was used. Both extracts were combined and extracted using HCX (200 mg, 10 ml) solid phase extraction columns. Drugs were eluted from the column using methylene chloride:isopropanol:NH_4OH (78:20:2, v/v/v). Extracts were evaporated to dryness, and derivatized with HFBA (50 µl). HFBA was evaporated and ethyl acetate (25 µl) was added. A Hewlett Packard GC-MS system comprising a 6890 GC and a 5973 MSD (CI with methane) was operated in SIM mode with splitless injection. For CLO *m/z* 315 and 279, for 7ACLO *m/z* 461 and 463, and for D_5 diazepam *m/z* 289 ions were monitored. Standard curves for CLO (10-400 pg/mg) and 7ACLO (1.0-20 pg/mg) were prepared by spiking aliquots (50 mg) of negative hair and had correlation coefficients of 0.998 and 0.989, respectively. In addition, two levels of control hairs were prepared for CLO and 7ACLO. All method validation parameters were within acceptable limits.

7ACLO was detected in hair of six out of ten volunteers. In two cases 7ACLO appeared in hair 3 days after CLO intake and remained detected for the entire 28-day study period (3.6-8.4 pg/mg and 2.7-3.0 pg/mg), and in two subjects 21 days later (4.9 and 2.7 pg/mg, and 1.2 and 23 pg/mg). In two volunteers 7ACLO was detected only on day 28 (1.8 and 3.3 pg/mg). CLO was not detected in any of the samples.

Key Words: Drug-Facilitated Sexual Assault, Date-Rape Drugs, Clonazepam, Solid Phase Extraction, NCI-GC-MS

Methamphetamine and Driving Impairment Revisited

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We have previously reported on the circumstances of traffic accidents involving drivers who test positive for methamphetamine (Logan BK, J For Sci, 1996;41(3):457-64) . These have invariably shown driving dominated by drive-off-the-road type accidents, where the driver crosses the median or centerline, or the fog line, and collides with other vehicles or fixed objects (87% of cases), or high speed, high risk driving (10% of cases) often involving police chases. The former driving pattern is most likely a result of fatigue and sleepiness resulting from withdrawal from the stimulant after excessive use. The latter effects are those more often associated with abuse of central nervous system stimulants. One of the limitations of that earlier study were the numerous cases in which multiple drugs other than amphetamines were present, such as cannabinoids, alcohol, and other CNS depressants. We have reviewed a further fifty cases of arrested drivers who tested positive for amphetamines alone, and evaluated the circumstances of the accident or driving that led to the arrest. Thirty of these cases included a complete drug recognition evaluation (DRE) exam, and the physiological findings in these cases are considered in the context of the blood drug concentrations. These cases confirm that methamphetamine abusers are vulnerable to impairment from their drug use once the stimulant effects of the drug have worn off, and withdrawal, characterized by inattentiveness, fatigue, extreme sleepiness, and depression. This group of cases however reveals more driving behaviors associated with cognitive and psychomotor impairment, which cannot necessarily be attributed to fatigue.

Keywords: Driving, Drug recognition, Methamphetamine

Uncertainty in Analytical Measurements, Implications for Forensic Toxicology

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Analytical toxicologists are coming under increased judicial pressure to demonstrate the quality of their results and in particular to demonstrate the fitness of the data for the decision by providing a measure of the confidence. This measurement confidence is expected to include the degree to which a result would be expected to agree with other results, normally irrespective of the methods used. To date, however, most emphasis for establishing this measurement confidence has been on showing the precision and bias of the results by using a specific method. This emphasis has led to the use of "official methods" to fulfill the requirements for measurement confidence while using internal quality control procedures, proficiency testing and accreditation as an aid in establishing traceability of the method to a given standard. There is now an additional way to demonstrate the degree in which a result, irrespective of the method, would be expected to agree with other results and that is measurement uncertainty.

This presentation will provide the essential tools for determining the total measurement uncertainty in an analytical measurement, examples of uncertainty measurements for analytical toxicology, and how to use uncertainty measurements for the judicial decision process.

Key Words: Uncertainty in Analytical Measurements; Measurement Quality Assurance

Postmortem Oxycodone Observations in North Carolina Deaths— State Statistics, Tissue Distributions, and Possible Trends

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Oxycodone is a semisynthetic opioid analgesic widely utilized in the United States for the management of moderate to moderately severe pain. It has been clinically available in an immediate release formulation since the early 1900s and as a controlled release formulation since the mid 1990s. Observations of recent years suggest that new trends in abuse may be emerging. For example, several deaths have occurred when the decedent reduced the oxycodone pill to a readily injectable solution. Nontolerant individuals are combining oxycodone with other "party-type" drugs to increase their euphoria, especially the sustained release form of oxycodone (Oxycontin®). Recent reports indicate a steady increase in both the medicinal and illicit use of oxycodone. Given these national trends and our own observations, the Office of the Chief Medical Examiner (NC-OCME) retrospectively reviewed all oxycodone-related deaths in the State of North Carolina between 1999 and 2000.

The incidence of oxycodone related deaths in North Carolina increased during the investigated time period. Out of 8,791 death investigations performed by NC-OCME in 1999, oxycodone contributed to 26 deaths (0.29 %) as the primary agent or a contributing agent. In 2000, 61 of 9,274 deaths (0.66%) were attributed to oxycodone. Thus, the percent increase in oxycodone related deaths from 1999 to 2000 was 228%.

Data for the distribution of oxycodone in postmortem fluid and tissue are limited. One of the objectives of this survey was to establish postmortem reference ranges that could be internally employed in our office. Of the 1999 and 2000 NC- OCME cases, a total of 114 cases were evaluated based on the presence of oxycodone. Deaths were categorically separated into two groups- deaths in which oxycodone was not listed as contributing to the cause of death (Non-CoD) and those in which oxycodone contributed to death (CoD), either exclusively or in combination with another agent or medical condition. The blood and tissue concentrations are indicated below with the number for each case group and the number for each specimen type reported in parentheses:

Oxycodone Cases	Central Blood (mg/L)	Peripheral Blood (mg/L)	Liver (mg/kg)	Urine (mg/L)	Gastric (mg Total)
Non-CoD (27)	0.053-4.0 (23)	0.044-0.89 (4)	1.9 (1)	0.17-1.7 (2)	2.0 (1)
CoD (87)	0.030-17 (74)	0.058-6.0 (28)	0.10-1.8 (11)	0.65-5.6 (6)	12 (1)

Postmortem redistribution of oxycodone was evident by an average central-to-peripheral blood concentration ratio of 2.5 ± 2.8 (range: 0.52-12; n=23). In most cases, the blood concentration was higher or equivalent to the liver concentration with an average blood-to-liver ratio of 2.0 ± 2.4 (range: 0.61-9.4; n=12).

Hence, this retrospective study indicates that oxycodone use is on the rise in the State of North Carolina. New findings with oxycodone suggest that toxicological interpretation may be more complex than previously indicated. Comparisons of blood and tissue concentrations demonstrate that postmortem redistribution of oxycodone occurred in a large number of cases. Blood and tissue concentrations should be carefully evaluated in light of the anatomical findings at autopsy and the drug use history to determine if oxycodone contributed to the death.

Key Words: oxycodone, postmortem, statistics

A study of alcohol pharmacokinetics of local Chinese in Hong Kong

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In drink driving cases, forensic toxicologists often confront with the requests for providing back-calculated alcohol levels of the drivers at the time of accident and refuting any of their inconsistent statements using the hip-flask defense tactics. So far, such calculations were based on data collected from Caucasians and their applicabilities to the local Chinese in Hong Kong had been challenged in courts. This study was designed to study the alcohol absorption profile and its rate of elimination of the local Chinese after consumption of different alcoholic drinks including beer, wine and spirit. With the Evidential Breathalysers (Alcotest 7110), the breath alcohol levels of over 200 healthy volunteers were measured after they finished drinking. The blood alcohol level was then calculated by assuming the breath to blood conversion factor of 1 : 2300. Information on the type and volume of alcoholic drinks consumed, age, sex, drinking habit, body weight, and drinking on empty stomach or after meal were recorded for each volunteer. The findings indicate that the Widmark's factor for Caucasians is generally applicable to local Chinese in estimating the peak alcohol level especially after the consumption of beer on empty stomach. Various factors affecting the absorption and elimination of alcohol are also discussed.

Key Words : alcohol pharmacokinetics, elimination rate, breath alcohol

Factitious Pediatric Acetaminophen Overdose in Siblings

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Two half brothers presented to a pediatric emergency department with jaundice and were subsequently admitted to the ICU with impending hepatic failure. The older child was symptomatic first and was much sicker on presentation. The boys were 14 months and 3 years old. Each had no previous history of illness and they both were up to date on vaccinations. The 14 month old had a febrile illness for 7 days that was presumed to have come from his older brother who had been sick for 2 weeks. The older child was seen at a pediatrician's office and was treated with amoxicillin for pharyngitis with fever. He had a positive monospot test. The older child had been icteric for 7 days and the family had just noticed icterus in the face of the younger child the day before admission to the pediatric hospital. The older child had transaminase values above 10,000, an INR of 3.8 and a total bilirubin of 9.There was no spontaneous bleeding. The younger child had transaminase values in the low thousands and a normal INR. The older child was toxic appearing with volume depletion, anorexia, malaise and vomiting. The younger child was not dehydrated and was taking po fluids well. Both children had received around the clock (four times per day) acetaminophen for at least a week. There was some confusion regarding the dose and it was possible that they had been receiving approximately 2 times the recommended dose for 3 to 7 days. Acetaminophen levels in the Emergency Department were drawn. The 3-year-old child had received a po dose of acetaminophen in the ED prior to a level of 17 and the level was 2.0 for the 14-month-old boy. A toxicology consultation was called for 'rule out acetaminophen toxicity leading to liver failure'. A diagnostic test was performed. The older child had an acute related donor liver transplantation. Neither child was thought to have significant acetaminophen poisoning. The epidemiology of acetaminophen poisoning in children will be briefly discussed along with the differential diagnosis of acute liver failure.

Key Words: acetaminophen, liver failure, jaundice

ANALYSIS OF AMITRIPTYLINE AND NORTRIPTYLINE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY USING POSITIVE CHEMICAL IONIZATION

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Amitriptyline (AMI) and Nortriptyline (NORT) continue to be detected in toxicology samples collected from medical examiner's cases. Most gas chromatography-mass spectrometry (GC-MS) procedures that analyze AMI and NORT utilize electronic ionization (EI). The EI mass spectrums of AMI and NORT show extensive fragmentation with prominent base peaks at m/z 58 and m/z 44 respectively. Many other compounds have similar EI mass spectrums. To improve specificity, a GC-MS method using positive chemical ionization (PCI) was developed. The method used liquid-liquid extraction with butyl chloride as the solvent. Deuterated analogues of AMI (AMI-d_3) and NORT (NORT-d_3) were used as internal standards. Following extraction, the extracts were derivatized with trifluoroacetic anhydride to improve the chromatography of NORT. The extracts were analyzed using an Agilent 5973 GC-MSD instrument and a 15 M X 0.32mm dimethylpolysiloxane capillary column. The PCI conditions used 200° C as the ionizer temperature and ammonia as the reagent gas. Because PCI is a more gentle ionization, the mass spectrum exhibited prominent protonated molecular ions (MH^+) for AMI (m/z 278), AMI-d_3 (m/z 281), NORT-TFA (m/z 377), and NORT-d_3-TFA (m/z 380). The assay was linear from 25 ng/mL to 1000 ng/mL. The extraction recovery was 63% for AMI and 53% NORT at 100 ng/mL. Intra-assay and inter-assay accuracy and precision were evaluated at 40 ng/mL, 100 ng/mL, and 500 ng/mL. In the accuracy evaluation, the AMI and NOR concentrations within 8% of target. The intra-assay and inter-assay coefficient of variations were less than 6 %. The TFA derivatives were stable at least 24 hours at room temperature. In this method, the monitoring of the higher molecular weight ions generated by PCI enhanced the specificity of identifying AMI and NORT. The method was used to identify and quantitate AMI and NORT in blood, urine and gastric samples submitted from medical examiner's cases.

Key Words: Mass Spectrometry, Positive Chemical Ionization, Amitriptyline